CAROTENOID PROFILES IN RELATION TO MATURATION, MOULTING, FOOD AND HABITAT IN THE INDIAN SPINY LOBSTER *PANULIRUS HOMARUS* (LINNAEUS, 1758)

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सारांश

कवचप्राणियों में प्राकृतिक वर्णकता के लिए कारक घटक है करोटिनोइड। कवचप्राणियों में लैंगिक चक्र कवच निर्मोचन, खाद्य आवास जैसे घटकों के अनुसार करोटिनोइड वर्णकों की मात्रा में परिवर्तन और पुनःवितरण हो जाता है। *पैनुलिरस होमारस* में पूर्व निर्मोचन, पश्च निर्मोचन और निर्मोचन अवस्थाओं के बीच करोटिनोइड के परिवर्तनों पर अध्ययन किया गया। निर्मोचन के पूर्व की अवस्था में करोटिनोइड की सान्द्रता उच्चतम देखी गई (होपाटोपानक्रियास में 774 मैक्रोग्राम/ग्राम, बहिः कंकाल में 725 मै.ग्रा/ग्रा. तथा पेशी में 133 मै.ग्रा/ग्राम)। निर्मोचन के बाद की अवस्था में करोटिनोइड की सान्द्रता निम्नतम थी। निर्मोचन के दौरान करोटिनोइड वर्णकों में 66% की कमी आकलित की गई। पूर्व निर्मोचन अवस्था में बहिःकंकाल और हेपाटोपानक्रियास में ऐस्टाजैन्थिन का स्तर उच्चतम देखा गया लेकिन पश्च निर्मोचन की अवस्था में इन ऊतकों में ऐस्टोजैन्थिन की सान्द्रता में तेज घटती हुई।

अंडाशय परिपक्वन के वक्त विभिन्न ऊतकों में करोटिनोइड की सान्द्रता में उल्लेखनीय परिवर्तन देखा गया। अपरिपक्व अंडाशय में करोटिनोइड का स्तर निम्न था (128 मै. ग्रा/ग्रा.) और पूर्ण रूप से परिपक्य अंडाशय में इनकी सान्द्रता में 512 मै.ग्रा./ग्रा. की वृद्धि देखी गई। अंडाशय की करोटिनोइड सान्द्रता की वृद्धि हेपाटोपानक्रियास के करोटिनोइड की घटती और बहिःकंकाल के करोटिनोइड सान्द्रता की वृद्धि के साथ संपात हुई। अंडाशय, हेपाटोपानक्रियास और बहिःकंकाल में करोटिनोइड मात्रा की घटती से अंडरिक्त अवस्था समझी जाती है। परिपक्वन के प्रारंभ में हेपाटोपानक्रियास में ऐस्टाजैन्थिन का जमाव हो जाता है और विटेल्लोजेनेसिस के दौरान ये अंडाशय की ओर जाते है। *पी. होमारस* में वर्णकता में प्रभावित होने वाले प्राकृतिक करोटिनोइडों पर अध्ययन किया गया। दिए गए चार खाद्यों में *मेटोपेनिअस डोबसोनी* और हरित शंबु पेनी विरिडिस खिलाए गए *पी. होमारस* में उत्कृष्ट वर्णकता देखी गई। *डोनाक्स कुनीटस* से खिलाए गए महाचिंगटों में सब से कम वर्णकता पाई गई। *डी. कुनीटस* को *स्पाइरुलीना* और *हीमटोकोकस पल्वियालिस* से समृद्ध करके *पी. होमारस* को खिलाया गया। इसके परिणाम से व्यक्त हो गया कि *पी. होमारस* की वर्णकता समृद्ध खाद्य से बढ़ायी जा सकती है। संपोषण पर किए गए अध्ययनों से व्यक्त हो गया कि स्पाइरुलीना से खिलाई गई सीपियां *एच.पल्वियालिस* से खिलाई गई सिपियों की अपेक्षा *एच. होमारस* को देने के लिए अच्छी है। वर्णकता में आवास के प्रभाव पर किए गए अध्ययनों से मालूम पडा कि प्रग्रहण की स्थिति में वर्णकता में पालन टैंकों के रंग का सीधा प्रभाव पड़ता है। उपर्युक्त चार विभिन्न रंगों (नीला, काला, ट्रान्स्लूसेन्ट और सफेद) में काले टैंक में पालन किए गए महाचिंगटों में उत्कृष्ट वर्णकता दिखाई पड़ी।

ABSTRACT

Carotenoids are responsible for natural pigmentation in crustaceans. The changes in the amount and redistribution of carotenoid pigments in crustaceans depend on several factors including sexual cycle, moulting, food and habitat. Carotenoid changes in *Panulirus homarus* were studied during thc premoult, postmoult and intermoult stages. Highest carotenoid concentration was recorded in the premoult stage (774µg/g in hepatopancreas, 725µg/g in the exoskeleton and 133 µg/g in the muscle). During postmoult stage the carotenoid concentration was lowest. Moulting accounted for 66% loss in carotenoid pigments. Astaxanthin levels were high in premoult stage in the exoskeleton and the hepatopancreas. During postmoult stage there was a sharp decrease in astaxanthin concentration in these tissues.

Significant differences in carotenoid concentration were recorded in different tissues during ovarian maturation. The carotenoid levels were low in the immature ovary (128µg/g) and in the fully mature ovary the concentration increased to 512µg/g. The increase in carotenoid concentration of the ovary coincided with a decrease in carotenoid concentration of hepatopancreas and increase in carotenoid concentration in the exoskeleton. Spent stage was characterized by decrease in carotenoid content in the ovary, hepatopancreas and exoskeleton. Astaxanthin was found to accumulate in the hepatopancreas during early maturation and during vitellogenesis they were mobilized to the ovaries. Different naturally occurring carotenoids were studied for their effect on pigmentation of *P. homarus*. Among the four feeds (*Donax cuneatus*, *Perna viridis, Metapenaeus dobsoni, Paphia malabarica*) used *P. homarus* fed on *M. dobsoni* and green mussel *P. viridis* had the best pigmentation pattern. The least pigmented lobsters were those fed on *D. cuneatus*. *D. cuneatus* was enriched with *Spirulina* and *Haematococcus pluvialis* and these were offered as food to *P. homarus*. The results revealed that pigmentation of *P. homarus* can be increased by enriched feed. Enrichment studies revealed that *Spirulina* enriched clams were superior to clams enriched with *H. pluvialis* when used as feed for *P. homarus*. The study on the influence of habitat on pigmentation pattern revealed that in captive conditions the colour of rearing tanks had a direct influence on pigmentation pattern. Among the four different colored tanks used in the study (blue, black, translucent and white) lobsters reared in black coloured tanks had the best pigmentation pattern.

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1. INTRODUCTION

"There was a time many years ago

Lobsters like all creatures great and small

Walked in a forward direction across the ocean floor

But now lobsters spend more time walking backward

How could such a thing come about?

These poor creatures have been poked

And prodded, studied examined and tested

By scientists for so many years

That they have learned to walk this way

Better to see and avoid the ever- present biologist "

D.E. AIKEN

'The Natural History of the American Lobster' - the tome on lobsters written in the late nineteenth century (Herrick, 1895), the citation above and the research papers published till date, all throw light on the research interest that lobsters have kindled in the biologist. With the fundamentals of lobster biology being revealed through scientific studies, lobsters have become the subject for physiological, biochemical and neurobiological research.

Lobsters are commercially important because they support a valuable fishery and are among the world's most valuable and highly priced seafood. Being the inhabitants of temperate and tropical seas they have graced the table

and delighted the palate of European aristocracy. Their value to humans is undeniable as a source of food as well as of revenue. Live spiny lobsters are sought in many parts of the world, particularly, Japan and South-East Asia. Besides being a relished food item, live spiny lobsters are gifted in Japan during traditional festivals, weddings and other auspicious occasions.

High demand for spiny lobsters in South-East Asian countries has generated considerable interest in capture as well as culture of these species. Palinurid lobsters contribute to 40.7% of world catch of lobsters. The world's largest producers of palinurid lobsters are Australia, New Zealand, Cuba, Brazil, South Africa, United States, India and Mexico (Bowen and Hancock, 1989).

Spiny lobster seed is yet to be produced commercially in the hatchery. The collection of puerulli (post larvae of spiny lobster) from the wild to sustain culture activities is not feasible. Non-availability of hatchery reared seed being the major 'bottleneck', spiny lobster culture practices solely depend on value addition by 'fattening' - the rearing of under sized juveniles to a desirable size or value addition of bigger lobsters by short term fattening to take them to the next higher grade. The increased demand in the foreign markets for live lobsters has lured aquaculturists to resort to fattening.

Colour is one of the major factors determining the acceptability as well as the marketability of seafood. During quality evaluation of seafood, any change in natural colouration is viewed negatively by consumers, thereby reducing the marketability of the product. Apart from the blackening caused by melanosis,

lack of adequate amount of carotenoids leads to changes in external colouration of crustaceans. Carotenoids and carotenoproteins are responsible for natural pigmentation in aquatic organisms. Most of the crustaceans including shrimps, crayfishes, crabs and lobsters are tinted by the presence of carotenoids.

External appearance such as shape and colour and sensory attributes like taste and smell are important factors in marketing of seafoods. The elite image and high market value of seafoods are in part due to the characteristic colour of the flesh (Christianen *et al.*, 1995a). It is not only important that fish have satisfactory flesh colour but also the colour has to be uniform. Colours most acceptable are red for salmonids (Hatlen *et al.*, 1997), greenish brown for *Penaeus monodon* (Menasveta *et al.*, 1993), red colour for whole cooked spiny lobsters *Panulirus homarus* (Zagalsky *et.al.*,1991) and pink / reddish yellow colour for red seabream, *Chrysophrys major* (Tomiyama, 1974). Like other fish and shellfish products, well-pigmented lobsters are commercially more valuable and acceptable because of the desirable red colouration when cooked. The attractive colouration of shellfish tends to fade in captivity (Tomiyama, 1974; Menasveta *et al.*, 1993) and lobsters are no exception to this phenomenon (Vijayakumaran and Radhakrishnan, 1984). Therefore, during culture conditions it is necessary to restore, improve or impart pigmentation to the cultured, organisms. Thus, it becomes imperative for the aquaculturist, to provide the right pigment for the organism to satisfy consumer expectations. It is in this context that carotenoids, apart from playing an important role in metabolism and immune enhancement, gain importance in aquaculture as a dietary supplement. Hence the present study has been undertaken to quantify

the carotenoids of the Indian spiny lobster *Panulius homarus* in the different tissues, study the variations occurring during moult cycle and maturation and to study the effect of dietary carotenoids in natural feeds in maintaining colouration. Studies on pigmentation profile of lobsters fed on carotenoid enriched natural feeds have also been attempted. The effect of the color of the rearing tanks on the pigmentation pattern of *P. homarus* was also studied using different coloured tanks.

2. REVIEW OF LITERATURE

Carotenoids are tetraterpenoid pigments of either aliphatic or acyclic structure composed of isoprenic residues which are linked so as to enable the centrally located methyl groups of the molecule to occupy positions 1:6 and the side methyl groups to be in positions 1:5 series of conjugated bonds (Karer and Jucker, 1948). Carotenoid research dates from the beginning of the nineteenth century with the isolation of bright red flat pigment cells from carrot roots. Wackenroder (1831) is credited with this discovery - the milestone which led to the wealth of data generated on carotenoid research till date. Discovery of polymorphism of carotenes and xanthophylls using chromatographic procedures (Tswett, 1908) and classical studies by Willslater and Mieg (1907) to determine the chemical formula of carotene and xanthophylls were important achievements in the first phase of carotenoid research. The second phase in the late 1920's was characterized by extensive use of chromatographic techniques for separation and spectral analysis of carotenoids to determine their structure. Use of sophisticated analytical techniques like infrared spectroscopy, proton nuclear magnetic resonance, and x-ray diffraction were major events in the third phase of carotenoid research.

Carotenoids are a class of compounds exhibiting diverse functions in plant and animal cells. These pigments in micro-organisms and algae perform a protective function with respect to photosensitizing effect of oxygen on cells. Being polyfunctional molecules, they are involved in mechanisms of electron transfer, photosynthetic and non-photosynthetic conversion of light energy to chemical energy of ATP and intramolecular linkage. Enhancement of

resistance of cells to ionizing radiation, ensuring their survival in an atmosphere of hydrogen sulphide, concentrated saline solution and natural thermal waters are other functions that these pigments perform.

The chain of unsaturated double bonds in the carotenoid molecule posseses properties of electron donor and acceptor system (Pullman and Pullman, 1963). Carotenoids accumulate oxygen under steady state conditions when the rate of oxygen intake is low. Increased oxygen utilization occurs as a result of cell activation. Oxygen deficit is made up for by its availability from depot systems. Carotenoids participate in the formation of energy generating intracellular organoid other than mitochondria, its function being energy supply to cells under hypoxia when normal mitochondrial activity is inhibited.

Carotenoids play a vital role in immune response of higher animals. They protect phagocytic cells from auto-oxidative damage, enhance T and B lymphocyte proliferative responses, stimulate effector T cell functions, enhance macrophage cytotoxic T cell and natural killer cell tumerocidal capacities and aid in production of interleukins (Andrianne,1989). Neutrophils, a major class of WBC's use reactive oxygen species and free radicals to kill phagocytic bacteria. Human neutrophils incubated with carotenoids and bacteria remain undamaged illustrating that these compounds have immunological properties. Crocetin, a water-soluble carotenoid has enhanced diffusive oxygen transfer in tissues including blood. It diminishes cholesterol and triglyceride concentration in sera of animals maintained on a cholesterol-enriched ration (De Luccio and Gainer, 1980), indicating a probable role for it in lowering coronary heart diseases.

These molecules enable adaptation of animal cells to hypoxic conditions and hence may prove to be anti carcinogenic (Karnaukhov, 1990), since tissue hypoxia is argued to be one of the principal causes of cancer (Smith and Kenyon, 1973). Carotenoids function as a chain breaking antioxidant in a lipid environment at physiologic oxygen partial pressures (Graham, 1989).

Besides the generalized functions discussed above, carotenoids have specific functions in finfish and shellfish. They are widely distributed in marine organisms especially in integuments and ovaries in finfish and shellfish and on the body surface of other invertebrates. They play an important role in protecting these organisms from injuries caused by free radicals and active oxygen radicals (Henk, 1999). Egg astaxanthin enhances chemotaxis of spermatozoa (Hartmann *et al.*, 1947). Deufel (1965) reported enhanced growth, maturation rate and fecundity in rainbow trout fed on diet supplemented with canthaxanthin. Fish eggs developing under highly oxygen depleted conditions tend to be highly pigmented than those under elevated oxygen conditions (Balon, 1979). Studies suggest that carotenoids perform respiratory function under conditions when oxygen is limiting as in fishes that hide or burrow their eggs.

Under intensive aquaculture conditions the fish and shellfish are predisposed to stress and subsequent infection by pathogens. Aimed at overcoming diseases, the industry is turning towards use of prophylactics and the therapeutic drugs. Immuno - enhancement by dietary manipulation mainly by supplementation of micronutrients offers a viable alternative to the use of

drugs, and subsequent development of drug resistant strains of pathogens. Of late, studies have proved that carotenoids have immuno-modulating action in fishes (Blazer, 1992; Lall and Olivier, 1993). Carotenoids have a potential as immuno stimulants in fish diets (Thompson *et al.*, 1995; Christianen *et al.*, 1995b; Amar *et al.*, 2000). β carotene has been found to increase spleen lymphocyte proliferation in parrot fishes (Tachibana *et al.*, 1997). In the rainbow trout dietary β carotene enhances immune responses by way of high serum complement activity and high levels of total plasma globulin (Amar *et al.*, 2000).

Studies on molluscan nerve cells have shown that carotenoids play an important role in the mechanism of these animals' resistance to environmental pollution and reduction of oxygen content in water (Karnaukhov *et al.*, 1977; Karnaukhov and Fedorov, 1977; Karnaukhov, 1979). The bluish colour due to carotenoprotein complex in pelagic invertebrates screens the internal organs against solar radiation (Herring, 1973). Carotenoprotein constitutes the main protein reserve of crustacean eggs (Ceccaldi, 1965) and in fishes, carotenoids are regular components of chromatophores.

Apart from their metabolic role as a dietary source of pro-vitamin A (Czeczuga and Czerpak,1976), the bright colouration imparted by carotenoids are important in meat colouration, courtship and sexual dichromatism. Recent studies have demonstrated a relationship between sexual colouration and carotenoid concentration in sticklebacks (Wedekind *et al.*, 1998). Males of *Gasterosteus aculeatus,* the three spined stickleback display red carotenoid based colouration during the breeding season and this plays an important role

in courtship and in the competition for breeding territories (Baube, 1997; Barber *et al.*, 2000). Brighter male sticklebacks were more successful in attracting females supporting the concept of a sexually dimorphic signal (Bakker and Mundwiler, 1994).

The ability to produce carotenoids is restricted to plants and microbes (Miki *et al.*,1982; Johnson and An,1991; Bjerkeng,1992). Hence the animal carotenoids are derived solely from food. Each animal species has its own carotenoid requirements and each tissue appears to have specificity in the assimilation of carotenoids because some are deposited unchanged, while some others are converted before being deposited. Crustaceans are incapable of *de novo* synthesis of carotenoids. (Goodwin, 1952a; Gillchrist and Lee, 1972; Herring, 1973; Zagalsky, 1976; Dall *et al.*, 1995; Petit *et al.*, 1998), but they have the ability to transform the pigments of their diet to endogenous forms (Goodwin, 1952a). Depending on the source of their origin, crustacean carotenoids are of two types; those derived from food and those obtained after the transformation from their precursors. Based on their chemical structure, crustacean carotenoids can either be esterified, non-esterified or at times they can even occur as protein complexes. The esterified forms are generally present in all organs and tissues, but in most crustaceans, protein complexes contribute to a wide variety of colourations. Crustaceans represent the class of animals where carotenoids are abundant. The carotenoids found in Crustacea are listed in Table1. The carotenoids and their relative proportions occurring in different organs and tissues fluctuate according to the species. The nature and concentration of pigments may also vary according to the geographic distribution of animals.

TABLE 1. CRUSTACEAN CAROTENOIDS

COMMON NAME	CHEMICAL NAME
Major Carotenoids	
β Carotene	β, β - carotene
Astaxanthin	3, 3' – dihydroxy – β, β-carotene-4, 4'-dione
Echinenone	β, β - caroten – 4 – one
Phoenicoxanthin	3-hydroxy – β, β - carotene – 4, 4' – dione
Canthaxanthin	β, β - carotene – 4, 4' – dione
Cryptoxanthin	β, β - caroten – 3 – o1
Zeaxanthin	β, β - carotene – 3, 3' – diol
Isocryptoxanthin	β, β - caroten – 4 - o1
Lutein	β, ε - carotene – 3, 3'diol
Minor Carotenoids	
Crustaxanthin	β, β - carotene – 3, 3', 4, 4' – tetrol
Violaxanthin	5,6, 5', 6' – diepoxy-5, 6,5', 6' – tetrahydro- β, β -carotene – 3,3' – diol
Idoxanthin	3, 3', 4' – trihydroxy - β, β,- Caroten – 4 – one

In regard to the tissue distribution of carotenoids in Crustacea, the major tissues involved are the carapace, hepatopancreas, ovary and muscle. The carotenoids present in the carapace are generally unesterified forms and protein complexes. The protein complexes are responsible for imparting the concerned colour to the animal. The hepatopancreas plays a major role in the absorption of carotenoid from food. Changes in the carotenoid content of hepatopancreas in Crustacea have been observed during vitellogenesis and ecdysis. The carotenoids from food pass through the gut wall into the body fluids and are distributed to other tissues and organs.

Major factors that affect the pigmentation pattern in crustaceans include ecological conditions, food and physiological status of the animal. Changes in pigmentation pattern occur throughout the life cycle of crustaceans and the major physiological changes include those occurring during maturation and moulting.

Ovarian development in crustaceans is accompanied by physiological and biochemical changes in the hepatopancreas and the ovary. Ovaries in spiny lobsters are paired cylindrical rods of tissue located beneath the heart, dorsolateral to the alimentary tract. Consequent to the developmental changes during the gonadotrophic cycle, changes occur in pigmentation pattern of ovaries. They become coloured during vitellogenesis due to development of oocytes. The major pigments in ovaries and eggs are carotenoids (Cheesman *et al.*,1967; Goodwin,1984; Vincent *et al.*, 1988; Matsuno and Hirao,1989). Immature white ovary in spiny lobsters changes to bright orange, later to a

darker orange colour during vitellogenesis and finally to brick red colour on maturity (Fielder, 1965; Berry,1971). During vitellogenesis most of the carotenoids of ovigerous individuals occur in the eggs. The protein complexes, in particular a lipoglycocarotenoprotein, constitute the crustacean lipovitellins. Various complexes result in different colouration depending on the species and stage of development.

Carotenoids in the egg binds to lipoprotein. Nature of carotenoid functioning as prosthetic group determines the colour pattern of eggs. Astaxanthin as prosthetic group imparts blue/ green colouration to the eggs of the lobster *Homarus gammarus* (Kuhn and Sorensen ,1938). Canthaxanthin results in a green colouration in anastrocans (Zagalsky,1976) and orange red colour in the eggs of brine shrimp, *Artemia salina* (Warner *et al.*,1972). Yellow colour in the eggs of crab *Callinectes sapidus* is the result of ß- carotene functioning as prosthetic group (Kerr, 1969).

Carotenoids and carotenoproteins of ovaries of few marine invertebrates have been characterized. Quantitative and qualitative analyses of whole carotenoids have been done in the mussel *Mytilus edulis* (Campbell *et al.*, 1967; Campbell, 1970), the sea urchin *Stronglyocentrotus drobachinensis* (Griffith and Perrott, 1976) and *Pseudocentrotus depressus* (Tsushima *et al.*, 1997). However studies on carotenoid composition of decapod ovaries have been limited and fragmentary (Goodwin, 1952b; Fox, 1976; Miki *et al.*, 1982; Dersan Kour and Subramoniam, 1992; Dall *et al.*, 1995).

Rapid development and pigmentation of ovaries in crustaceans indicate either a sudden increase in intake of dietary carotenoids or an appreciable carotenoid reserve or both (Dall *et al.,* 1995). Crustaceans undergoing maturation possibly mobilize carotenoids from flesh and selectively transfer them to the gonad and the exoskeleton. Changes in carotenoid profiles suggest a possible role for it in reproduction (Tacon, 1981; Torrisen,1984). Ability to store carotenoids changes throughout the life cycle. Fingerlings have limited capacity for carotenoid deposition in flesh, while significant amounts are deposited in the skin in fishes (Storebakken and No, 1992). Sexual maturation can have a profound influence on ingestion, absorption and metabolism of carotenoid pigments (Hatlen *et al.*, 1996) as evidenced by redistribution of carotenoids from flesh to skin and eggs in fishes (Steven, 1949; Choubert and Blanc, 1989; Torrisen, 1989).

Sexual maturation in salmonids leads to a decrease in the muscle carotenoid content (Hatlen *et al.*, 1996). This fall in carotenoid content is ascribed to mobilization of pigments from flesh to skin and ovaries, since carotenoid content of these tissues are known to increase at the time of sexual maturation (Steven, 1949; Crozier, 1970; Kittahara, 1983; Bjerkeng, 1992; Choubert *et al.*,1994). Addition of astaxanthin to practical broodstock diets improves ovarian development and spawning of *Penaeus monodon* (Panganthion *et al.*,1998). High levels of astaxanthin and predominance of the free form in the ovary and its poor disposition in the hepatopancreas of *Penaeus monodon* fed with astaxanthin supplemented diets suggest its involvement in shrimp reproduction. Shellfish furnish specific carotenoids to

their ovaries especially during maturation by rejecting some and assimilating and modifying others among the dietary carotenoids.

Studies on carotenoid composition of different tissues of sea urchin revealed the highest level of carotenoids in the gonads, the maximum titre being reported during spawning season (Tsushima *et al.*,1997). Ovaries are found to have higher affinities for carotenoid deposition in salmonids (Torrisen and Torrisen,1985). This, together with the mobilization of flesh astaxanthin and transportation and deposition in ovaries during sexual maturation, supports the hypothesis of function of carotenoids in reproduction or in the early life stage.

Besides maturation, moulting is another important physiological process affecting the carotenoid profile in crustaceans. Crustacean life cycle is marked by tegumental rearrangement associated with important biochemical modifications. The periodic removal of cuticle is accompanied by the activation of pigment transfer from internal organs to the newly forming teguments. These pigmentary movements imply qualitative as well as quantitative variations within different tissues during the moult cycle. There appears to be a paucity of information on the relationship between moulting and pigmentation in Crustacea. Studies on these lines have been fragmentary and only a few references are available (Lenel,1961; Negre,1974, 1978; Castillo *et al.*,1988; Petit *et al.*,1997,1998). Dietary astaxanthin modifies exuviation frequencies, shortens moult cycle and hastens postlarval development in *Penaeus japonicus* (Petit *et al.*,1997).

Carotenoproteins, both inside and outside chromatophores, play an important part in the general colouration of crustaceans, especially in the decapods (Goodwin, 1960; Cheesman *et al*, 1967). Since fishes and crustaceans are unable to synthesise carotenoids *de novo,* their pigmentation pattern results from the pigments present in the diet and accumulated in the tissues (Goodwin, 1951; 1954; Fox, 1957; Hata and Hata, 1973; Foss *et al.*, 1984; Choubert and Blanc,1989;Choubert and Heinrich,1993; Britton *et al.*,1995; Borhan *et al.*,1995). Astaxanthin and canthaxanthin are the carotenoid pigments added to the feed in the form of industrially synthesised compounds (Chang,1999; Barbosa *et al.*,1999; Hoppe *et al.*,1999; Buttle,1999). Astaxanthin (3, 3'- dihydroxy-4, 4' diketo-β, β–carotene) is one of the major pigments concentrated in the shells of crustaceans and represents 60-80% of the total pigments (Ishikawa *et al.* ,1966; Katayama *et al.*,1972; Chien and Jeng,1992; Torrisen,1995).

Metabolism of carotenoids is species specific. Seabreams belong to the sea- bream type which cannot convert β carotene, canthaxanthin, or zeaxanthin to astaxanthin but can only transfer these carotenoids to tissues from feeds. Unlike seabreams, crustaceans belong to the prawn type which converts β carotene, canthaxanthin and zeaxanthin to astaxanthin, exceptions being *Artemia, Daphnia* and certain malacostracan isopods.

The metabolic pathway for *Panulirus japonicus* has been established as follows (Katayama *et al.*, 1973):

β-carotene → isocryptoxanthin → echinenone → 4, hydroxyechinone → canthaxanthin → 3, hydroxycanthaxanthin → astaxanthin.

The general pathway for carotenoid metabolism in Crustacea starts with β carotene, the end product being canthaxanthin or astaxanthin. Crustaceans can be classified into two types depending on the site of metabolism of β–carotene (Castillo *et al.*, 1982). The first category being prawn type where the conversion from β-carotene to astaxanthin occurs mainly in the hepatopancreas as in the case of the prawn *Penaeus japonicus* (Katayama *et al.*, 1972). The second group embraces animals converting β carotene to echinenone in the hepatopancreas, with all further transformations upto astaxanthin taking place in other tissues like epidermis as in the spiny lobster *Panulirus japonicus* (Katayama *et al.*, 1972). Diversity exists among crustaceans in the metabolic pathway caused mainly by the absence or presence of particular intermediate products. These differences are probably due to particular enzymatic systems involved in metabolism or merely due to the availability of carotenoids in the natural environment (Goodwin, 1951).

Comparative studies of available natural carotenoids from marine sources as pigmenters have been done by various authors (Peterson *et al.*, 1966; Zagalsky, 1967; Lambertsen and Braekkan,1971; Spinelli,1979; Simpson and Kamata,1979; Torrisen *et al.*, 1981; Torrisen ,1982; Choubert and Luquet,1983 ; Choubert and Blanc,1989 ; Johnson *et al.*,1993;). Carotenoids of plant origin have been tested as pigment sources in aquatic feeds (Peterson *et al*, 1966; Ahmed 1966; Tanaka *et al.*,1976; Matty and Smith, 1978; Choubert,

1979 ; Johnson *et al.*,1980; Tacon and Jackson,1985; Choubert *et al.*, 1992; Barbosa *et al.*,1999) and these include the green algae *Haematococcus pluvialis*, Alfalfa, the single cell protein *Spirulina,* and the yeast *Phaffia rhodozyma*. Besides carotenoids of plant and animal origin, synthetic products like carophyll pink, carophyll red, zeaxanthin, and lutein have been tested as dietary supplements in pigmentation studies. (Deufel, 1965; Schmidt and Cuthbert, 1969; Malak *et al.*, 1975; Choubert and Luquet, 1979; Torrisen *et al.*, 1981; Torrisen, 1982; 1986; 1995; Foss *et al.*, 1984; Storebakken *et al.*, 1986; Choubert and Heinrich, 1993).

Carotenoid metabolism and subsequent deposition in Crustacea have been found to be under neuroendocrine control (Castillo *et al.*, 1982). The neurosecretary complex of eyestalks as well as activity of androgenic gland appear to have a marked influence on various physiological cycles related to carotenoid metabolism (Castillo *et al.*, 1982). The colour of the surroundings is considered to have a marked influence on pigmentation patterns in aquatic organisms.

3. MATERIALS AND METHODS

3.1. Collection and maintenance of animals

Live spiny lobsters, *Panulirus homarus,* for the study were collected from Kasimedu and Kovalam, two fishing villages of Madras coast from skin divers and from bottom set gillnet catches. Lobsters caught by skin diving were preferred because these animals were healthier and handling stress was minimal. Feeding experiments were conducted on lobster which were acclimatized for two to three weeks in the laboratory. During the acclimatization period, the lobsters were maintained in 1-ton fiberglass tanks filled with filtered seawater. Continuous aeration was provided by an air pump. The lobsters were fed *ad libitum* on the clam *Donax cuneatus* (with shells) during the acclimatization period. The water quality parameters maintained were: salinity 32 ± 0.5 ppt; dissolved oxygen 4 ± 0.2ml/l; pH 8 ± 0.5. Hundred percent water exchange was done daily to ensure good water quality and thereby to reduce stress on the experimental animals.

3.1.1. Estimation of carotenoids during moult cycle in *Panulirus homarus*

Three moult stages viz., premoult, postmoult and intermoult were chosen to study the changes in carotenoid concentration during moult cycle. Identification of moult stages was done by microscopic examination of pleopods based on the method described by Drach and Tchernigovtzelt (1967) (Table 2). For each stage, a minimum of six samples was taken for carotenoid determination. The samples had a weight range of 150 to 300 g each. The lobsters collected were sacrificed for the study immediately after identifying the moult stages and the tissues processed for analysis.

TABLE 2. CLASSIFICATION OF MOULT STAGES OF *PANULIRUS HOMARUS*

STAGE	MORPHOLOGICAL FEATURES
Premoult (Stage D_0 - D_4)	Determined by the degree of development of new setae. Epidermis withdraws from the cuticle at the base of setae. Circular invagination forms in the tissue at the base of each old setae followed by the secretion of new setae. Appearance of longitudinal decalcified line in branchiostegiate area.
Postmoult (Stage A- B)	Cuticle becomes soft during the early stages of postmoult denoting the secretion of the post exuvial endocuticle.
Intermoult (Stage C)	Formation of calcified endocuticle becomes complete and formation of innermost layer of the noncalcified endocuticle begins.

TABLE 3. CLASSIFICATION OF MATURITY STAGES OF *PANULIRUS HOMARUS*

STAGE	MORPHOLOGICAL FEATURES
Stage 1 (Immature)	Ovary white in colour, flattened and strap like with granular appearance.
Stage 4 (Ripe\Mature)	Ovary swollen, orange red in colour and occupying all available space in body cavity. Fresh sperm mass present in the sternum.
Stage 5 (Spent)	Ovary pale cream in colour. Residual ova present.

3.1.2 Estimation of carotenoids during maturation in females of *Panulirus homarus*

Female lobsters were collected, and their maturity stages were identified as described by Berry (1971). Three maturity stages corresponding to stage 1 (immature), stage 4 (mature or ripe) and stage 5 (spent) were chosen to study the variations in carotenoid concentration during maturation cycle. Details for identification of maturity stages are given in (Table 3). For each stage a minimum of six samples were taken for carotenoid determination. For the immature stage the samples had a weight range of 100 to 150 g each and for the mature and spent stages the weight range was 250 to 300 g each. The animals were sacrificed immediately after collection and tissues processed for carotenoid estimation. The tissues analysed were hepatopancreas, ovary, muscle and exoskeleton.

3.1.3. Effect of different diets on carotenoid concentration of *Panulirus homarus*

Lobster juveniles (100 to 150 g live weight) were used for the study. The acclimatized juveniles were divided into four groups; each containing three numbers reared individually in 30 litre capacity blue coloured plastic containers. Group A was fed with the razor clam *Donax cuneatus*, while groups B, C and D were fed with the green mussel *Perna viridis*, the textile clam *Paphia malabarica* and the prawn *Metapenaeus dobsoni* respectively. The food items were collected in fresh condition from the landing centre at Kasimedu and were frozen immediately with the shell. Two hours prior to feeding, only the required amount was weighed and thawed. The bivalves were shucked before feeding

while the prawn was fed with the shell. The animals were given a ration at 10% of body weight. The water was changed everyday in the evening just before feeding and the feed remains were removed the next day morning. The water quality was maintained at normal levels (salinity 32 ±0.5ppt; dissolved oxygen 4±0.2ml/l; pH 8 ± 0.1) during the course of experiment. Feeding experiments were conducted for a period of 79 days so that two moults were obtained for each lobster. On the day prior to moulting, the animals cease feeding. Feeding on such days was suspended to prevent deterioration in water quality. At the end of the experiment, the animals were sacrificed and the tissues (hepatopancreas, muscle and exoskeleton) were analysed for total carotenoids. Photographs were taken to illustrate the difference in colouration when animals were fed with different feeds.

3.1.4 Carotenoid concentration of *Panulirus homarus* fed with carotenoid enriched natural diet.

During the process of fattening, a common phenomenon observed was a gradual decrease in the colour pattern. Lobsters fed on the clam *Donax cuneatus* exhibited a marked decrease in colouration after a period of 100 days. These lobsters were used to study whether there would be enhancement in colouration by supplementing the natural diets with carotenoids. These pale lobsters (100 to 150g) obtained after feeding with *D. cuneatus* were divided into 3 groups; control group, group A and group B. The control group was fed with *D. cuneatus* without enrichment, group A was fed with *D. cuneatus* enriched with *Haematococcus pluvialis* and Group B was fed with *D. cuneatus* enriched with *Spirulina*. In each group three animals were maintained individually in separate blue containers.

Since the compounded feed is not preferred by the lobsters, the carotenoids had to be incorporated in the natural feeds. Initial experiments were conducted to determine the best method of carotenoid incorporation in the diets. Direct injection of carotenoids into the lobsters was found to result in their death. Meat sausages injected with carotenoid solution also proved futile due to the leaching out of the solution into the water medium during the feeding process. As a result the water quality deteriorated, leading to the death of the lobsters. Intramuscular injection of carotenoids dissolved in vegetable oil into the natural feeds did not yield success, as most of the injected solution leached out into the water medium while the lobster used its appendages to capture the feed. The best alternative found was to enrich the live clams *Donax cuneatus* with algae containing carotenoids. (Table 15a). Enrichment of *D. cuneatus* was done by exposing the clams to different concentrations of algae (0.5mg/ml,1mg/ml,1.5mg/ml and 2mg/ml) with high carotenoid content for 1 hour (Table 15c). Being a filter feeder the clam accumulated the algae into their system. The clams thus enriched were fed to the lobsters.

To determine the ideal time duration for enrichment, the live clams *D. cuneatus* were exposed to filtered sea water containing algae at a concentration of 2mg/ml (Table 15b). Clams were sacrificed at every one hour interval, for six hours. The carotenoid concentration was determined by spectrophotometry. The ideal time duration for enrichment was found to be 1 hour. Carotenoid sources used for enrichment were single cell protein *Spirulina,* and the green algae *Haematococcus pluvialis* containing 2.8%

astaxanthin obtained from Parry Nutraceuticals. Continuous aeration was given to keep the algae in suspension during the enrichment period. Pale lobsters fed on live clam *D. cuneatus* served as the control group. The first experimental group consisted of three lobsters fed on clams enriched with *Spirulina*, and the second group was fed on clams enriched with *H. pluvialis*. Feeding was done in the evenings immediately after enrichment of feed in the evenings. At the end of feeding experiments, which lasted for a month, the animals were photographed and then sacrificed and tissues analysed for total carotenoids. Water quality maintenance and feeding levels were the same as in the experiments with natural feeds.

3.1.5. Influence of the colour of rearing tanks on pigmentation of *Panulirus homarus*.

Of the several factors contributing to the pigmentation in spiny lobsters, a neuro-endocrine control on pigmentation has been reported, which enables them to adjust the colour based on the colour of the surroundings. Hence studies were undertaken to find out the effect of different coloured rearing tanks on pigmentation in *P.homarus*.

Four different coloured tanks were chosen for the experiment, viz., black, and white, translucent and blue. After acclimatization for a period of three weeks, the animals, (100 to 150 g) were transferred to experimental tanks of 250-litre capacity. Each tank contained four animals. Since the tanks were not covered, the lobsters in all the treatments were exposed to natural daylight and the only difference was the colour of the rearing tanks. During the rearing period the animals were fed on the clam *D. cuneatus*. Fifty percent water

exchange was done everyday to ensure good water quality. The experiment lasted for 90 days after which the animals were sacrificed and the tissues subjected to carotenoid analysis.

3.2. Determination of total carotenoids

Estimation of total carotenoids was done by the method described by Kelly and Harmon (1972). To 50 g of tissue (hepatopancreas, ovary, muscle, exoskeleton) 10 g of silica gel and 100ml of 75% acetone were added. After ensuring complete mixing, it was filtered through a glass funnel till dripping ceased. The container and filter were rinsed with 50 % acetone. The leftovers of the tissue in the funnel was blended with 15 to 20 g anhydrous sodium sulphate and 100 ml of 1:1 2 - propanol: chloroform mixture. Filtration and re-extraction with 50 ml acetone was done so as to obtain a colourless residue in the funnel. 2 - propanol: chloroform mixture was used as the rinse solution. Filtrate was transferred to 500 ml round bottom flask. To this 5 ml of chloroform was added and then evaporated to dryness. The residue was dissolved in pure cyclohexane and filtered through sodium sulphate in a glass funnel. Sodium sulphate was washed with cyclohexane to remove all traces of colour. The filtrate was diluted to 100ml and read at 474 nm on Cecil 373 linear read out spectrophotometer. Total carotenoids present in the sample was calculated using an extinction coefficient of 2150. The calculation used was as follows:

$$\text{Gram pigment / gram wet tissue} = \frac{(A) * (100ml)}{100 * (\text{weight of tissue}) * (d) * 2150}$$

d = cell width; A =Absorbance at 474 nm

All extractions were carried out in the dark to prevent influence of light on carotenoid pigments. During the process of extraction, nitrogen gas was passed through the samples to prevent the influence of atmospheric oxygen.

3.3. Qualitative analysis of carotenoids using HPLC

Extraction procedure

The procedure described by Kelly and Harmon (1972) was used for extraction of carotenoids from samples. The extract thus obtained was stored under nitrogen at – 20° C in a deep freezer.

HPLC separation of carotenoids

HPLC separation of astaxanthin and canthaxanthin, the major carotenoids in marine products, was carried out by slight modification of the procedure described by Bhaskar Achary *et al.* (1995). Modification in procedure was in the extraction step where cyclohexane was used as solvent for reconstitution instead of acetone. The chromatographic system consisted of Shimadzu model LC6A equipped with system controller, SCL a variable wavelength detector, SCL 6A an integrator, C-R3A chomatopack and a stainless steel 15cm * 4.6mm separation column (Shobdex ODS18, 5 micrometer particle, Dupont). By injecting 10 micro litre of sample extract on to the HPLC column, isocratic separation of carotenoids was accomplished with mobile phase consisting of acetonitrile / dichloromethane / methanol in 70:20:10 v/v proptions pumped at a flow rate of 1 ml/minute . The effluent was monitored at 474 nm and the sensitivity was 0.01 AUFS. External standards used were carophyll red and carophyll pink obtained from Hoffman

Roche, Norway. The HPLC was calibrated daily by injecting a full loop (20 micro litre) of standards. The retention times of the standards and samples were compared. Peak identification was based on the retention times of samples with that of the standards (Figure 2a). Astaxanthin standards dissolved in cyclohexane had retention time of 2.5 minutes and canthaxanthin had retention time of 1.5 minutes.

3.4. Statistical analysis

Statistical tests such as mean, standard deviation, test of significance, ANOVA and Duncans multiple range test were done following Snedecor and Cochran (1967).

4. CAROTENOID CHANGES IN *PANULIRUS HOMARUS* DURING THE MOULT CYCLE

Crustacean life cycle is characterized by moulting, a unique feature where growth is manifested in stanzas. In decapod crustaceans, this periodic removal of cuticle is one of the major energy demanding processes, besides vitellogenesis. Interrelationship between moulting and reproduction determines the pattern of oogenic cycle in females. Brachyurans have an intermoult period which is protracted to accommodate all ovarian activities including spawning and hatching of eggs from pleopods. Natantians are diecdysic in that the premoult period is longer than that of intermoult period. (Kaleemur Rahman and Subramoniam, 1989). Hence the entire ovarian growth cannot be completed within the intermoult period and is therefore extended to the premoult period. In spiny lobsters, the intermoult is long enough to accommodate one or more ovarian cycles, although premoult duration is generally as long as intermoult (Kaleemur Rahman and Subramoniam, 1989). The ovarian cycle may extend to premoult in spiny lobsters as moulting has been observed to take place shortly after the release of phyllosoma larvae in many cases (Vijayakumaran and Radhakrishnan, 1999).

Moult stage is characterized by morphological and physiological changes. The morphological and biochemical changes occurring during moulting have been studied in the clawed lobster *Homarus americanus* (Donahue, 1954; Aiken, 1973; Gilgan and Zink, 1975) and in palinurid lobsters (Scheer and Scheer, 1951; Schwabe *et al.*, 1952; Travis, 1954, 1955; 1957;

Dall, 1977; Radhakrishnan, 1989; Vijayakumaran, 1990). Generally, the biochemical constituents are accumulated during intermoult and premoult for subsequent utilization during ecdysis and growth (Drarch,1939; Andrews,1967; O'Connor and Gilbert,1968; Spindler – Barth,1976). In the homarid lobsters lipids are considered to be the organic reserves utilized during ecdysis. (Passano, 1960; Barclay *et al.*, 1983; Chang and O' Connor, 1983). Though the utilization of various biochemical constituents during moulting in palinurid lobsters have been studied (Drarch, 1939; Travis, 1955; 1957; Andrews, 1967; Dall, 1977; Radhakrishnan, 1989), there appears to be a paucity of information on the changes in the concentration of carotenoid pigments at various stages of the moult cycle. The present study attempts to find out the accumulation, changes and utilization of carotenoids at various stages of moult cycle in the Indian spiny lobster *Panulirus homarus*. Generally astaxanthin is the predominant carotenoid pigment in Crustacea which is responsible for the colour of exoskeleton. Astaxanthin supplemented diets were found to increase the moulting frequency and shorten the moulting cycle in the post larvae of *Penaeus japonicus* (Petit *et al.*, 1997). In view of the importance of astaxanthin, studies have been carried out to find out the changes in astaxanthin content, if any, occurring during the different moult stages in *P. homarus*

4.1. RESULTS

4.1.1. Carotenoid changes in hepatopancreas

Carotenoid concentration in the hepatopancreas of *P. homarus* during different moult stages showed significant variations according to the results

shown by anova and Duncans multiple range test. Carotenoid concentration of hepatopancreas in the intermoult stage was 328µg/g. It increased to 774µg/g in the premoult stage and after moulting the carotenoid concentration reduced to 288µg/g. Comparison of carotenoid concentration in the hepatopancreas between the three moult stages was done using Duncans multiple range test (Appendix I). Significant difference was observed between the three stages with the highest concentration in the premoult stage followed by intermoult and postmoult stages respectively. Astaxanthin concentration of hepatopancreas during intermoult stage was 117µg/g. It increased to 465µg/g in the premoult stage followed by a sharp decrease to 65µg\g during postmoult stage (Fig.2) (Table 6).

4.1.2. Carotenoid concentration of exoskeleton

As in the hepatopancreas, the carotenoid concentration in the exoskeleton showed significant variations during moult stages ($P<0.05$) (Table7). At intermoult stage, the carotenoid concentration of exoskeleton was 512µg/g. During premoult, the concentration increased to 725µg/g and in the postmoult stage, the concentration dropped to 235µg/g. Carotenoid concentration of exuvia was 119.75µg/g. The results of Duncans multiple range test showed that the carotenoid concentration in the exoskeleton was highest in the premoult stage and the lowest in the postmoult stage. Astaxanthin concentration of exoskeleton during intermoult stage was 267µg/g. It increased to 515µg/g in the premoult stage followed by a decrease to 96µg/g during postmoult stage (Fig.3).

TABLE 4. Carotenoid content (µg/g) in different tissues of *P. homarus* during different moult stages

Tissues	Moult stages		
	Mean ± SD. n = 6		
	Intermoult	Premoult	Postmoult
Hepatopancreas	328 ± 17.2	774 ± 13.3	288 ± 11.6
Exoskeleton	516 ± 27.3	725 ± 38.8	235 ± 21.6
Muscle	115 ± 4.4	133 ± 4.9	92 ± 3.9

TABLE 5. Carotenoid content (mg/100g body weight) in different tissues of *P. homarus* during moult stages

Tissues *	Moult stages Mean ± SD n=6		
	Intermoult	Premoult	Postmoult
Hepatopancreas	0.98 ± 0.017	2.32 ± 0.013	0.86 ± 0.011
Exoskeleton	26.11 ± 0.027	36.69 ± 0.038	11.89 ± 0.021
Muscle	3.27 ± 0.004	3.78 ± 0.004	2.61 ± 0.003

* Weight of Tissues when expressed in 100 g

Hepatopancreas - 3 g

Exoskeleton - 50.6 g

Muscle - 24.8 g

Fig 1 Quantitative changes in carotenoid reserves of various tissues during moult cycle in *P. homarus* (values expressed in 100g live weight)

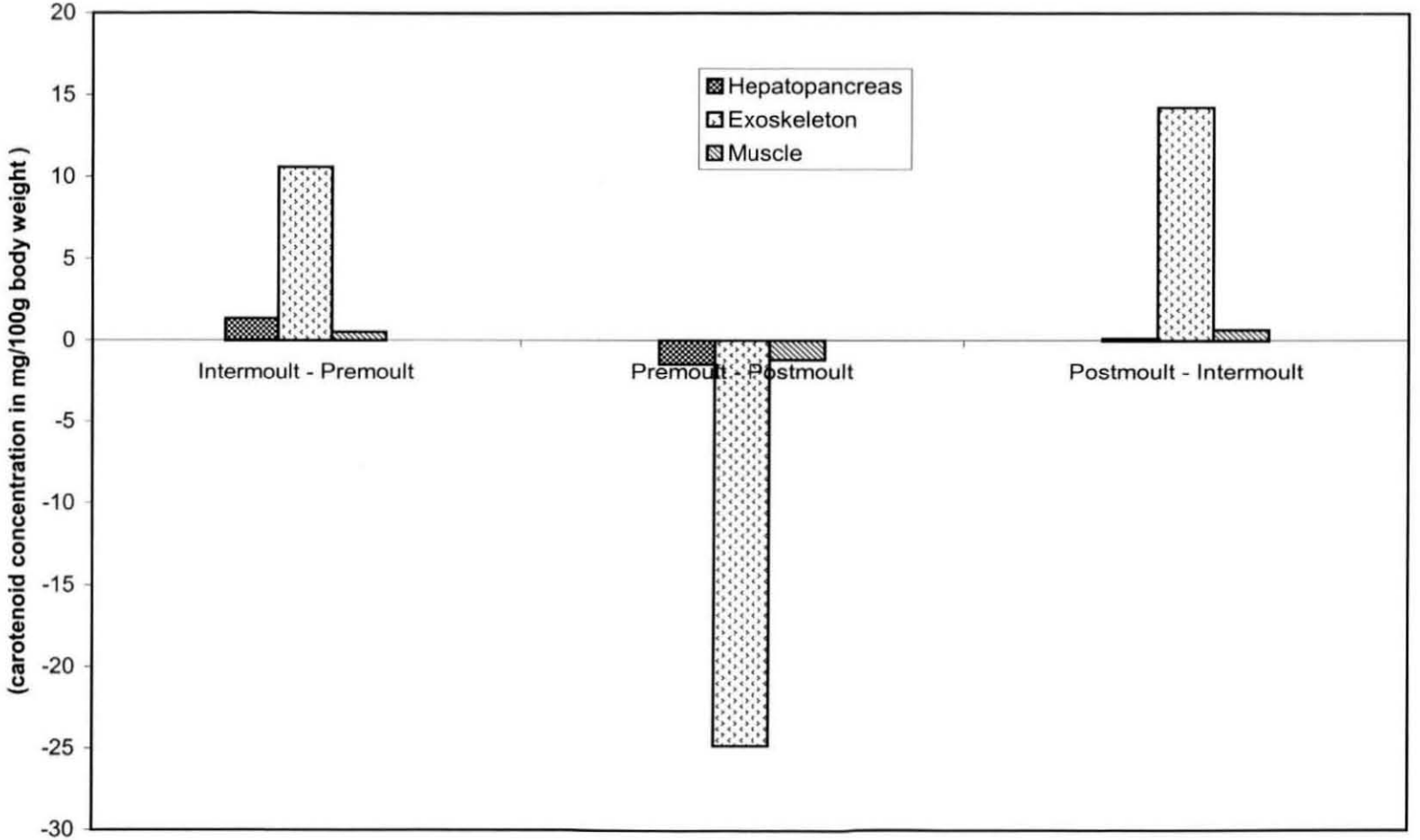


Fig.2 Chromatographs of standards

ASTAXANTHIN

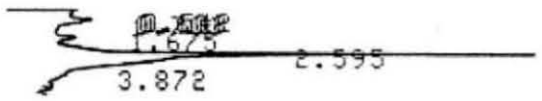
CHROMATOPAC		C – R3A	FILE	0		
SAMPLE	NO	0	METHOD		41	
REPORT	NO	305				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.500	47739	S		100	
	TOTAL	47739			100	

CANTHAXANTHIN

CHROMATOPAC		C – R3A	FILE	0		
SAMPLE	NO	0	METHOD		41	
REPORT	NO	336				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.587	55584	V		100	
	TOTAL	55584			100	

Fig.2 a. Chromatographs showing carotenoid profiles of hepatopancreas during different moult stages

INTERMOULT – HEPATOPANCREAS



CHROMATOGRAM 9 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 337

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.595	7981	S		100	
	TOTAL	7981			100	

PREMOULT – HEPATOPANCREAS

CHROMATOGRAM 9 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 336

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.587	55584	V		100	
	TOTAL	55584			100	

POSTMOULT – HEPATOPANCREAS

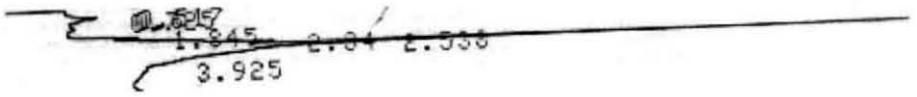
CHROMATOGRAM 9 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 319

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.415	9106			100	
	TOTAL	9106			100	

Fig.3. Chromatographs showing carotenoid profiles of exoskeleton during different moult stages

INTERMOULT – EXOSKELETON

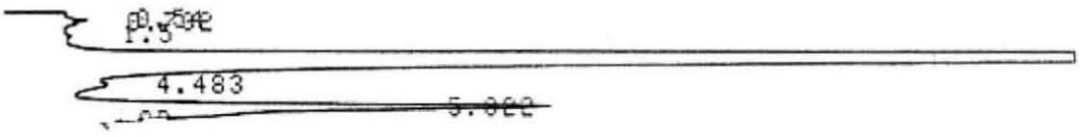


CHROMATOGRAM 12 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 339

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.342	3257	V		22.0382	
2	2.538	11522	SV		77.9618	
	TOTAL	14778			100	

PREMOULT – EXOSKELETON

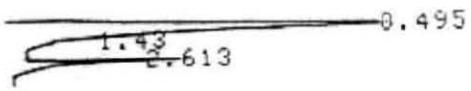


CHROMATOGRAM 7 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 335

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.565	52351			49.3917	
2	2.88	40519	SV		38.2289	
3	5.822	13121			12.3794	
	TOTAL	105991			100	

POSTMOULT – EXOSKELETON



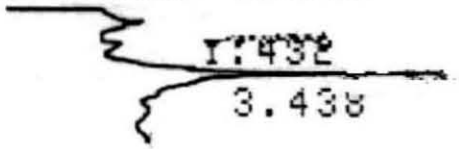
CHROMATOGRAM 10 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 339

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.495	11907	S		86.1786	
2	2.613	1910			13.8213	
	TOTAL	13817			100	

Fig.4. Chromatographs showing carotenoid profiles of muscle during different moult stages

INTERMOULT – MUSCLE

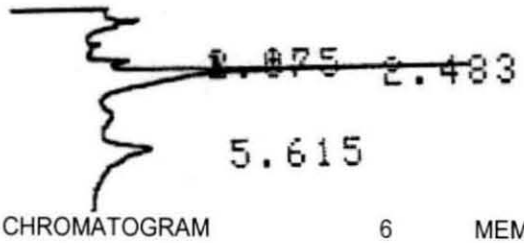


CHROMATOGRAM 15 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 342

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.542	3144	V		100	
	TOTAL	3144			100	

PREMOULT – MUSCLE

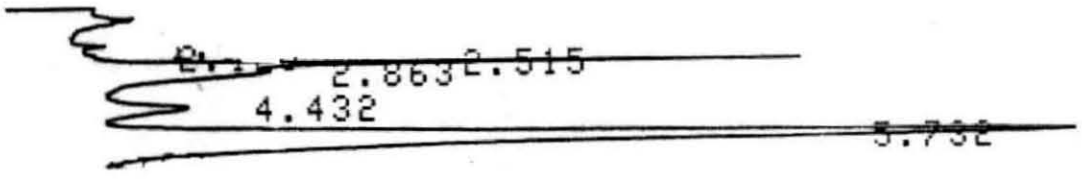


CHROMATOGRAM 6 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 334

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.483	3091	V		100	
	TOTAL	3091			100	

POSTMOULT MUSCLE



CHROMATOGRAM 5 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 333

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.515	4436	V		13.1521	
2	2.863	3698	V		10.9623	
3	4.432	1764	V		5.2306	
4	5.732	23833			70.655	
	TOTAL	33732			100	

TABLE 6. Astaxanthin content (µg/g) in different tissues of *P.homarus* during moult cycle

Tissues	Moult stages*		
	Intermoult	Premoult	Postmoult
Hepatopancreas	117	465	65
Exoskeleton	267	515	96
Muscle	28	20	45

* Standard deviation was not given since the samples were pooled.

Table 7. Anova for the carotenoid concentration during moult stages

HEPATOPANCREAS

Source of Variation	SS	df	MS	F
Between Groups	874667.1	2	437338.6	2143.8
Within Groups	3060	15	204	
Total	877727.1	17		

EXOSKELETON

Source of Variation	SS	df	MS	F
Between Groups	725677.8	2	362838.9	399.2
Within Groups	13633.3	15	908.9	
Total	739311.1	17		

MUSCLE

Source of Variation	SS	df	MS	F
Between Groups	8485.778	2	4242.9	9.4
Within Groups	6797.1	15	453.1	
Total	15282.9	17		

4.1.3. Carotenoid concentration of muscle

The carotenoid concentration in the muscle during intermoult stage was 115µg/g. It increased to 133µg/g in the premoult stage and in the postmoult stage the carotenoid concentration decreased to 92 µg/g. During the three moult stages the carotenoid concentration in the muscle was significantly different as shown by anova. Group wise comparison by Duncans multiple range test showed that there was significant difference between the treatments. Astaxanthin content of muscle was 28µg/g in the intermoult stage. At premoult stage the astaxanthin concentration decreased to 20µg/g and later at postmoult stage the concentration of astaxanthin registered a steep increase to 45µg/g(Fig.4).

4.2. DISCUSSION

The changes in the amount and distribution of carotenoid pigments in Crustacea is dependent on moulting (Goodwin, 1960; Menasveta *et al.*,1993). In general, moulting diminishes in senescent individuals and stops in certain species, when the animal reaches a limit of size, vitellogenesis and hibernation being the interruptions in the moult cycle.

Hepatopancreas plays a major role in the absorption of carotenoid pigments from food. During late premoult and early postmoult this organ exhibits a reduced size. In the postmoult stage the hepatopancreas becomes less coloured in *P.homarus*. Late premoult stage is characterised by cessation of feeding and also by the accumulation of nutrients in the hepatopancreas

(Radhakrishnan, 1989). Among the nutrients, lipid levels showed siginificant increase during premoult stages. Lipid accumulation had been reported to occur in the hepatopancreas of *P. homarus* during late premoult stage. (Radhakrishnan, 1989). Increase in lipid content of hepatopancreas has been reported in *Cancer pagurus* (Paul and Sharp, 1919; Renaud,1949), *Panulirus argus* (Travis,1955), *Orconectes virilis* (O'Connor and Gilbert, 1969), *Pachygrapsus marmoratus* (Lautier and Lagarrigue, 1976) and *Penaeus indicus* (Read and Caulton,1960). It is likely that stored lipids act as reserves during late premoult stage when the animal does not feed. During premoult stages a build up of carotenoids occur in the hepatopancreas along with other nutrients for further mobilization during the ensuing phenomenon of ecdysis. This could be a valid explanation for the increase in carotenoid concentration of hepatopancreas during premoult and the decrease during postmoult in *P.homarus.* Intermoult cycle in Brachyurans and all decapods is the stage where tissue growth and mineralization of cuticle are achieved (Castillo *et al.*, 1982). During the period between late intermoult and early premoult, lipid level in the hepatopancreas increases and the organ becomes highly coloured in *P.homarus.* The results of the present study showed that the rate of increase in carotenoid concentration in the hepatopancreas was maximum during the period from intermoult to premoult suggesting that there is a progressive accumulation of carotenoids in the hepatopancreas, which may be utilized for ecdysis.

Observation on the changes in carotenoid pigments in the hepatopancreas of *P. homarus* during different moult stages have shown a

similar trend to those reported in the hermit crab *Clibanarius erythropus* (Castillo *et al.*,1988). In *C. erythropus*, the hepatopancreas had the highest carotenoid concentration of 500μg/g in the premoult stage. The postmoult stage showed the lowest caroteniod concentration of 250μg/g and the carotenoid concentration in the intermoult stage was 460μg/g.

Carotenoids form complexes with proteins and are responsible for the colour of the exoskeleton in crustaceans (Goodwin, 1952b). Carotenoprotein complexes are regular components of the chromatophores (Castillo *et al.*, 1982). Studies on exoskeletal carotenoids have revealed the presence of astaxanthin as the only carotenoid in the tissue in the Norwegian lobster, *Nephrops norvegicus* (Goodwin and Srisukh, 1949). The premoult period is characterized by quantative variations within the tissues. Accumulation of protein occurs during premoult and intermoult in palinurid lobsters (Scheer and Scheer 1951; Radhakrishnan, 1989). During premoult stage an increase in carotenoid concentration was observed in all the tissues analysed. The newly forming teguments appear in the premoult stage and hence there is a chance for the pigments being mobilized from the exoskeleton to the newly forming teguments. This mobilization should result in the decrease of carotenoid pigments, but in *P.homarus* at late premoult stage the concentration increases. High values of exoskeletal carotenoids during premoult have been reported in the hermit crab *Dardanus arrosor* (Castillo, 1972) Clibanarius *erythropus* (Castillo *et al.*, 1988) and in the crab Pachygrapsus *marmoratus* (Negre, 1974). In this study, the carotenoid concentration in the exoskeleton of *P. homarus* during premoult stage was 725μg/g. An increase of about 200μg/g occurred in

the premoult stage in comparison with the concentration during intermoult stage. The increase in carotenoid concentration in the exoskeleton and hepatopancreas during premoult stages shows that the lobster equips itself with the necessary pigments from its diet for further mobilization to the newly forming teguments and also to restore the pigments lost during ecdysis. Hence it can be assumed that transfer of pigments from hepatopancreas to exoskeleton for further mobilization to the newly forming pigments can be the cause for increase in exoskeletal carotenoid concentration during premoult.

The postmoult stage is characterized by decrease in carotenoid concentration in all the tissues analysed. This could be due to the losses during moulting. Moulting accounts for nearly 66% loss in the carotenoid concentration in *P.homarus* in the present study. Moulting loss of pigments in this study was in line with the results for *Panulirus interruptus* in which 60 to 80% loss was reported. (Fox, 1953; Menasveta *et al.*, 1993). Limited supply of carotenoids during late premoult due to cessation of feeding coupled with losses in carotenoid pigments in exuvia and also the mobilization to the newly formed teguments can be the other reasons for lower carotenoid values during postmoult stage. In the present study the carotenoid concentration of all the tissues of *P.homarus* increases considerably at intermoult when compared with postmoult stages. As the animal resumes feeding, the intake of dietary carotenoids could have contributed to the overall increase in pigment concentration during intermoult stage. Though an increasing trend in the carotenoid concentration was observed during the period from postmoult to intermoult, the percentage increase was not as high as that occurring during

intermoult to premoult stage. From premoult to postmoult stage, a decline in the carotenoid concentration occurred. Rate of increase in the carotenoid concentration in the hepatopancreas was maximum during the period from intermoult to premoult, suggesting a progressive accumulation for further utilization during ecdysis. Percentage increase in exoskeletal carotenoid concentration was highest during the period from postmoult to intermoult. Accretionary growth and hardening of post exuvial layers occur at this stage. Synthesis and elaboration of organic matrix and elaboration of constituents for hardening of exoskeleton occur by mobilization of protein and chitin. Hepatopancreas plays a significant role in this process. Carotenoids being associated with proteins, it is possible that these pigments are channelized through the hepatopancreas to the exoskeleton resulting in high values of these pigments. Carotenoprotein and lipoprotein intermediates are involved in the transfer of pigments from the hepatopancreas to the exoskeleton via the haemolymph (Lee and Gilchrist, 1972; Czeczuga and Krywuta, 1981; Castillo *et al.*, 1982).

Astaxanthin and canthaxanthin are the major pigments responsible for colouration in salmonids. The level of astaxanthin in tissues depends on the physiological status of the animal. Moulting being a unique phenomenon in crustaceans, it is possible that these pigments play a significant role. Astaxanthin shortens moult cycle in penaeids (Petit *et al.* ,1997). With regard to qualitative analysis of carotenoids in *P.homarus* only two carotenoids namely astaxanthin and canthaxanthin were studied. Besides these two pigments, other pigments like lutein, echinenone and isocryptoxanthin may be present.

These pigments could not be studied due to the non-availability of standards. Astaxanthin levels in *P.homarus* were high in premoult stages in the hepatopancreas and exoskeleton. In the premoult stage astaxanthin represented 60% of the total carotenoids in the hepatopancreas and in the postmoult and intermoult stages the contribution was only 23% and 35% respectively. In the exoskeleton of *P.homarus* the percentage contribution of astaxanthin to the total carotenoids was 70% and this finding is in accordance with those reported in *C.erythopus* (Castillo *et al.*, 1988). The relatively lower levels of astaxanthin in the hepatopancreas in the postmoult stage clearly indicates that this pigment may be metabolised during the process of ecdysis. But further studies at the enzymatic level are required to confirm the above inference. During postmoult stages in *P. homarus* the astaxanthin levels were high in the muscle in comparison to the levels during premoult and intermoult stages. This could be due to resorption of astaxanthin from other tissues. The deposition of astaxanthin from dietary sources could be another cause.

Astaxanthin levels were high in premoult stages in the hepatopancreas and exoskeleton. During postmoult astaxanthin levels decrease drastically. This fall in astaxanthin levels in postmoult stages and increase in premoult stages indicate the relationship between moulting and carotenoid concentration. Canthaxanthin did not give significant peaks in the chromatograms probably due to the fact that it is below detectable levels. It can be concluded that astaxanthin has a major role in the physiology of lobsters.

Lobsters in the early postmoult stage with the soft exoskeleton are generally weak due to the loss of hard carapace, and also due to the limited supply of food during the late premoult stages. The energy loss for the process of moulting added to the above factors makes the animal weak and chances of mortality are high. Astaxanthin acts as oxygen scavengers, wherein the electrically charged oxygen molecules are captured and this antioxidant action prevents tissue damage (Graham, 1989; Henk, 1999). The high levels of astaxanthin in the tissues in the premoult stage and the drastic decrease in postmoult stage clearly shows that astaxanthin is utilized. It could be assumed that part of astaxanthin could be mobilized to the newly formed teguments while the rest would have been utilized as antioxidants to prevent possible tissue damage during ecdysis.

The chromatographs of exoskeleton and muscle show peaks other than astaxanthin, especially in the premoult exoskeleton and the postmoult muscle. These could not be indentified due to lack of standards but it deserves further elucidation.

The values of carotenoids in different tissues have been quantified in a uniform weight of hundred gram body weight and expressed in Table 5 and Fig.1. The total decrease from premoult to postmoult in hepatopancreas, exoskeleton and muscle are 1.46mg, 24.8mg and 1.17mg respectively. Alternatively it can be inferred that of the total loss of 27.43mg carotenoid during ecdysis of a hundred gram lobster, about 93% loss is from exoskeleton. Part of the loss is accounted in exuvia while the rest would have been utilized for the moulting process.

Moulting, the tegumental rearrangement occurring in crustaceans is associated with biochemical modifications and results in growth. Carotenoids are responsible for natural colourations in salmonids and in crustaceans. The results of the present study on the relationship of carotenoids to the moult stages in *P.homarus* have revealed that moulting is one of the factors, which determine the pigmentation pattern. In general the carotenoid concentration is highest in premoult stages. The intermoult stages had carotenoid concentration which were lower than the premoult but higher than postmoult stages. Considering the external colour early premoult and intermoult stages would be the ideal stages to market lobsters. Generally, the late premoult lobsters are not marketed live, since chances of mortality are high. It would be profitable to retain the postmoult stage lobsters and feed them with carotenoid rich diets to improve pigmentation patterns.

5. CHANGES IN CAROTENOID CONCENTRATIONS DURING OVARIAN MATURATION IN *PANULIRUS HOMARUS*

Study of reproductive physiology in crustaceans enables understanding of the intricate mechanisms involved in vitellogenesis, the mobilization of nutrient reserves from various tissues and their subsequent deposition in the ova. Mobilization of nutrient reserves from the storage organs like hepatopancreas to the ova determines the hatching rate and the survival of the larvae.

Hepatopancreas acts as the major nutrient storage organs in crustaceans. Significant increases have been reported in the level of protein and carbohydrates in the hepatopancreas of *P.homarus* during the process of maturation (Vijayakumaran,1990; Radhakrishnan, 1989). The total lipids in the ovary increase during sexual maturation in *Panulirus homarus* (Vijayakumaran,1990), in *Panulirus polyphagus* (George and Patel,1956) and in *Thenus orientalis* (Kaleemur Rahman and Subramoniam,1989) and are transferred to the mature ova. Spawning causes depletion of lipids in the ovary in crustaceans. Utilization of lipid resources stored in the hepatopancreas for gametogenesis has been documented in *P. homarus* (Vijayakumaran, 1990).

Carotenoids form part of lipid reserves in crustacean hepatopancreas and ovaries. They exhibit marked variations during the gonadotropic cycle and are reported to play a significant role in reproduction in animals (Matsuno, 1991). Deposition of carotenoid pigments in the ovary has been reported in

crustaceans and fishes (Tacon,1981). Hatching rates were higher for red seabream larvae fed with carotenoid supplemented diets. Enhanced growth, fecundity and maturation rates have been recorded in rainbow trout fed on diets supplemented with canthaxanthin (Gillchrist and Lee,1972; Tacon,1981). The degree of pigmentation determines the ability of fish larvae to tolerate adverse conditions such as low oxygen levels, elevated temperature and ammonia concentration (Mikulin and Soin,1975; Tacon,1981). Astaxanthin enhances the chemotaxis of the sperm of rainbow trout (Hartmann *et al*, 1947). During sexual maturation in salmonids considerable amounts of carotenoids are transferred to the eggs. The pattern of movement of carotenoids reported in chum salmon was from the muscle to the ovaries. (Ando, 1986).

Attempts have been made in the present study to evaluate the extent to which maturation affects pigmentation in *P.homarus*. Studies were conducted to find out the concentration and mobilization of carotenoids in various tissues (ovary, hepatopancreas, exoskeleton and muscle) during ovarian maturation. Evaluation of carotenoid changes during ovarian maturation were followed in three stages, Stage 1 (immature), Stage 4 (mature) and stage 5 (spent) (Table 3). All the lobsters chosen for the study were in the intermoult stage. The methods of tissue preparation and carotenoid analysis are described in chapter 2.

5.1. RESULTS

5.1.1. Carotenoid changes in the ovary

Ovarian carotenoid concentration during maturation in *P. homarus* showed significant variation ($P<0.05$) (Table 11). Carotenoid concentration was

TABLE 8. Carotenoid content (μg/g) in different tissues of *P. homarus* during maturity stages

Tissues	Maturity stages (Mean ± SD) n=6		
	Immature	Mature	Spent
Ovary	128 ± 2	512 ± 25	88 ± 2
Hepatopancreas	520 ± 16	320 ± 16	277 ± 18
Exoskeleton	330 ± 15	472 ± 20	219 ± 17
Muscle	125 ± 36	88 ± 5	140 ± 3

TABLE 9. Carotenoid content (mg/100g body weight) in different tissues of *P. homarus* at different maturity stages

Tissues *	Maturity stages Mean ± SD n=6		
	Immature	**Mature**	**Spent**
Ovary	0.19 ± 0.002	1.54 ± 0.025	0.13 ± 0.002
Hepatopancreas	1.97 ± 0.016	1.06 ± 0.016	0.77 ± 0.018
Exoskeleton	16.7 ± 0.015	23.9 ± 0.020	11.08 ± 0.014
Muscle	3.55 ± 0.036	2.50 ± 0.003	3.98 ± 0.003

* Weight of tissues when expressed in 100 g

Ovary
- Immature - 1.5 g
- Mature - 3 g
- Spent - 1.5 g

Hepatopancreas
- Immature - 3.79 g
- Mature - 3.3 g
- Spent - 2.8 g

Exoskeleton - 50.6 g

Muscle - 28.4 g

TABLE 10. Astaxanthin content (μg/g) in different tissues of *P.homarus* during maturity stages

Tissues	Maturity stages*		
	Immature	Mature	Spent
Ovary	37.2	60.8	21
Hepatopancreas	197	75	67
Exoskeleton	117	317	45
Muscle	51	39	65

* Standard deviation is not given since the samples were pooled.

TABLE 11. ANOVA FOR MATURITY STAGES

OVARY

Source of Variation	SS	df	MS	F
Between Groups	657907	2	328953.5	1438.8
Within Groups	3429.5	15	228.6	
Total	661336.5	17		

HEPATOPANCREAS

Source of Variation	SS	df	MS	F
Between Groups	200530.8	2	100265.4	337.3
Within Groups	4459.5	15	297.3	
Total	204990.3	17		

EXOSKELETON

Source of Variation	SS	df	MS	F
Between Groups	192219.4	2	96109.7	318.1
Within Groups	4532.17	15	302.1	
Total	196751.6	17		

MUSCLE

Source of Variation	SS	df	MS	F
Between Groups	8485.8	2	4242.9	9.4
Within Groups	6797.2	15	453.1	
Total	15282.9	17		

128µg/g in immature ovary. As the ovary matured, the carotenoid concentration increased to 512 µg/g and declined to the lowest value in the spent stage. Comparison of carotenoid concentration in the ovary between the three maturity stages was done using Duncans multiple range test (Appendix II). Significant difference was observed between the three stages with the highest concentration in the mature stage and lowest in the spent stage. Astaxanthin and canthaxanthin were the individual carotenoids estimated in the three maturity stages. Canthaxanthin was below detectable levels in all the stages. The rate of change of astaxanthin concentration in the ovary during maturation was more pronounced than the changes in total carotenoids. Astaxanthin content in the ovary during immature, mature and spent stages were 60.8µg/g, 372.µg/g and 21µg/g respectively. (Fig. 6). The chromatographs of mature and spent ovaries indicated other minor peaks which could not be detected for want of standards.

5.1.2. Carotenoid changes in hepatopancreas

Statistical analysis of carotenoid concentration in hepatopancreas during the different maturity stages showed significant variation ($P<0.05$) as shown by Anova and Duncans multiple range test. Hepatopancreas of immature stage had maximum level of carotenoids (520µg/g). Decline in the quantity of carotenoid content of hepatopancreas was significant at mature stage and more so in the spent stage (277µg/g). Astaxanthin levels recorded the highest value of 197µg\g in immature stage followed by a sharp decrease to 75µ/g in mature stage and a further decline to 67µg/g in spent stage (Fig. 7).

5.1.3. Carotenoid changes in the exoskeleton

Significant changes in the carotenoid concentration of exoskeleton was recorded during ovarian maturation ($P<0.05$). The exoskeleton of immature lobsters had a carotenoid concentration of 330µg/g. The carotenoid concentration increased to 472µg/g in mature stage and later decreased to 219µg/g in the spent stage. Results of Duncans multiple range test showed that carotenoid concentration was highest in the mature stage and lowest in the spent stage. Astaxanthin concentration was 117 µg/g in the immature stage, 317 µg/g in the mature stage and 45µg/g in the spent stage. (Fig. 8).

5.1.4. Carotenoid changes in the muscle

During the three maturity stages the carotenoid concentration in the muscle was significantly different as shown by anova. Group wise comparison by Duncans multiple range test showed that there was significant difference in the carotenoid concentration of the muscle between the three maturity stages. Carotenoid concentration of muscle was 125µg/g in immature stage and decreased to 88µg/g in the mature stage. There was an increase in concentration to 140µg/g in the spent stage. Astaxanthin concentration of the muscle in the immature stage was 51 µg/g. It decreased to 39 µg/g in the mature stage and increased to 65µg/g after spawning (Fig. 9).

5.2. DISCUSSION

Carotenoids in fishes are regular components of chromatophores. In salmonids these polyene pigments are responsible for brilliant breeding colourations (Crozier, 1970). Carotenoid functions have been ascribed to the

behavioral association during courtship and also to sexual dichromatism (Hubbs and Slavenhagen,1958). The changes in the distribution of carotenoids during the different maturity stages formed the basis of the hypothesis that carotenoids have a function in reproduction similar to those in fishes (Tacon,1981; Torrisen,1984). In the present study, the carotenoid concentration in the immature ovary is at the lowest level. When the ovary becomes mature there is a sharp rise in carotenoid content. During the maturing period the oocytes enlarge in size. The increase in the carotenoid concentration in the mature ovary can be regarded as an increase in the carotenoid content of each oocyte. The spent stage ovary recorded a decrease in carotenoid content. The movement of carotenoids from the ovary to the ova just before or during spawning could be the cause of depletion of carotenoids in the spent stage as drastic changes are reported in the chemical composition of just spawned egg compared to the mature ova in *P. homarus* (Vijayakumaran, 1990). This finding is in accordance with the reports in Salmonids, (Crozier, 1970; Torrisen,1985).

Crustaceans take up carotenoids from the diet and store them in the chromatophores. It is quite possible that along with the transfer of nutrient reserves, ovarian carotenoids are also furnished to the ova. So the non feeding stages during the early larval period have to depend entirely on the yolk reserves of carotenoids. The high level of astaxanthin (372µg/g) in the mature ovary of *P. homarus* bears evidence to the fact that shellfish furnish specific carotenoids to their ovaries by rejecting some and assimilating and modifying their dietary pigments (Miki *et al.*, 1982). Vincent *et. al* (1988) reported

Fig 5 Quantitative changes in carotenoid reserves of various tissues during ovarian maturation in *P. homarus* (value expressed in 100g live weight)

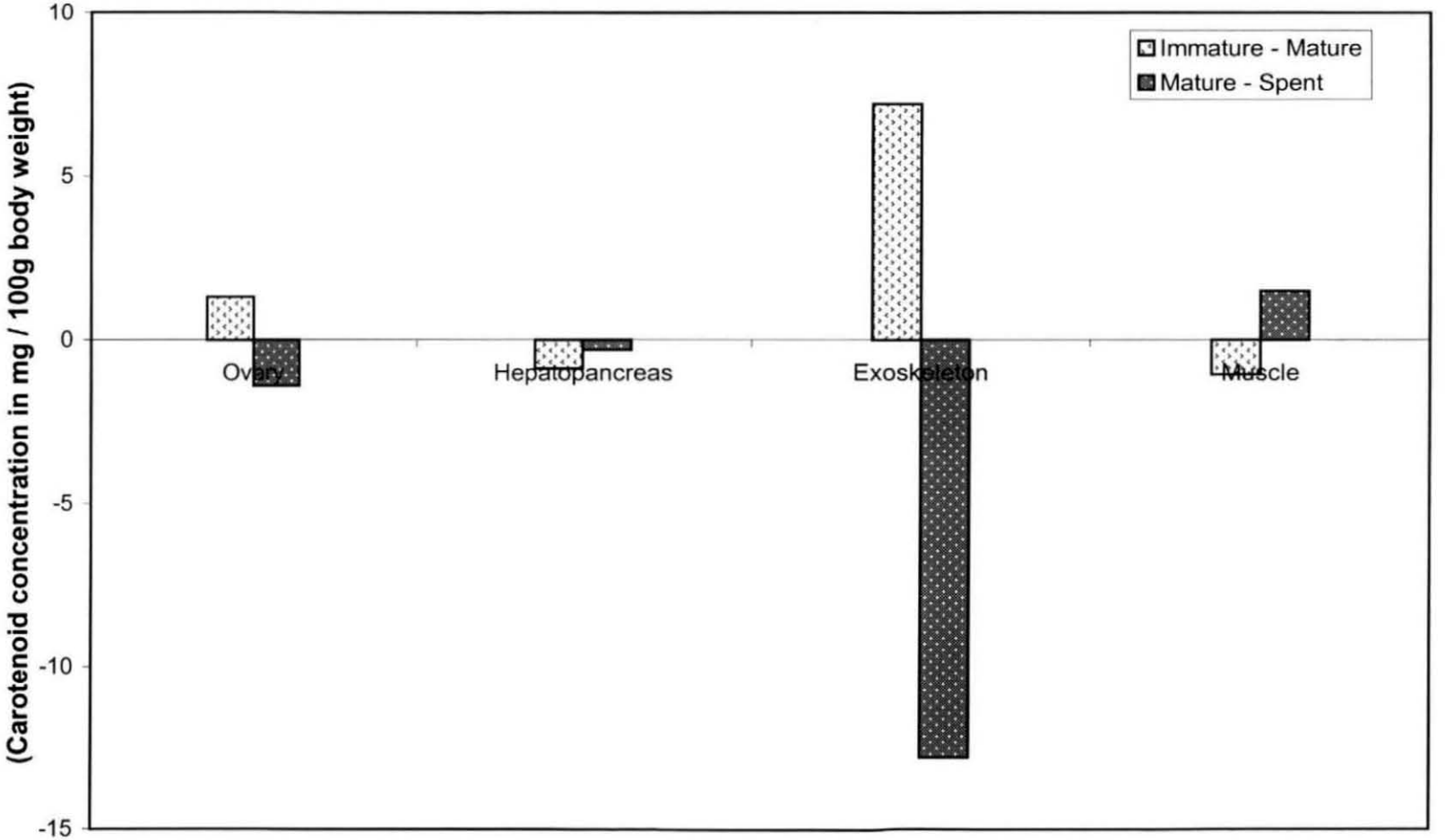


Fig. 6. Chromatographs showing carotenoid profiles of ovary during different maturity stages

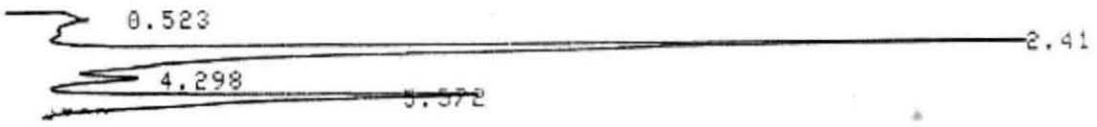
IMMATURE - OVARY

CHROMATOGRAM 10 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 321

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.633	7257	V		100	
	TOTAL	7257			100	

MATURE - OVARY

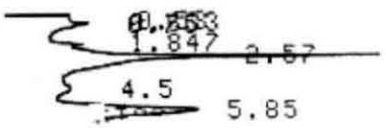


CHROMATOGRAM 5 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 316

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.523	2180			3.4535	
2	2.41	44425	S		70.3832	
3	4.298	1577	T		2.4992	
4	5.572	14936			23.6641	
	TOTAL	63118			100	

SPENT - OVARY



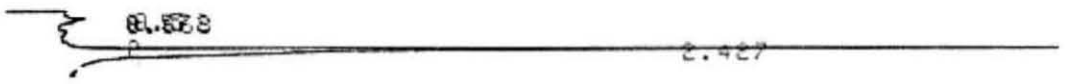
CHROMATOGRAM 13 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 340

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.57	6028			69.2747	
2	5.85	2674	V		30.7253	
	TOTAL	8702			100	

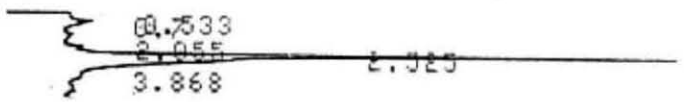
Fig.7. Chromatographs showing carotenoid profiles of hepatopancreas during different maturity stages

IMMATURE – HEPATOPANCREAS



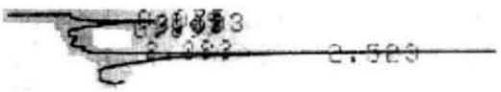
CHROMATOGRAM		6	MEMORIZED			
CHROMATOPAC		C – R3A	FILE		0	
SAMPLE	NO	0	METHOD		41	
REPORT	NO	317				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.427	13445	SV		100	
	TOTAL	13445			100	

MATURE – HEPATOPANCREAS



CHROMATOGRAM		15	MEMORIZED			
CHROMATOPAC		C – R3A	FILE		0	
SAMPLE	NO	0	METHOD		41	
REPORT	NO	326				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.525	8968	SV		100	
	TOTAL	8968			100	

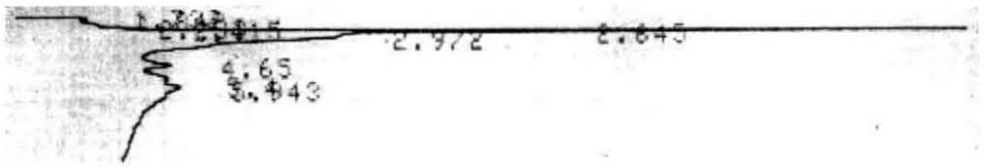
SPENT – HEPATOPANCREAS



CHROMATOGRAM		11	MEMORIZED			
CHROMATOPAC		C – R3A	FILE		0	
SAMPLE	NO	0	METHOD		41	
REPORT	NO	322				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.523	4629	V		100	
	TOTAL	4629			100	

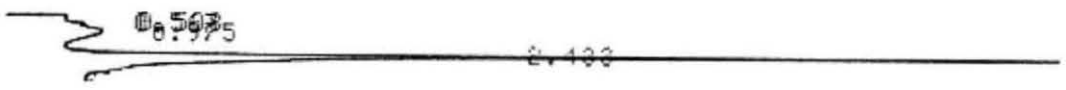
Fig.8. Chromatographs showing carotenoid profiles of exoskeleton during different maturity stages

IMMATURE – EXOSKELETON



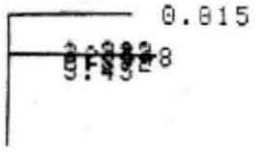
CHROMATOGRAM		14	MEMORIZED			
CHROMATOPAC	C – R3A		FILE	0		
SAMPLE NO		0	METHOD		41	
REPORT NO		323				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.645	8041	V		51.9201	

MATURE – EXOSKELETON



CHROMATOGRAM		7	MEMORIZED			
CHROMATOPAC	C – R3A		FILE	0		
SAMPLE NO		0	METHOD		41	
REPORT NO		318				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.975	1566	V		9.3554	
2	2.433	15172			90.6446	
	TOTAL	16738			100	

SPENT - EXOSKELETON



CHROMATOGRAM		17	MEMORIZED			
CHROMATOPAC	C – R3A		FILE	0		
SAMPLE NO		0	METHOD		41	
REPORT NO		328				
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.548	3095	V		45.3725	
2	2.892	2348	V		34.416	
3	3.45	1379	V		20.2114	
	TOTAL	6822			100	

Fig.9. Chromatographs showing carotenoid profiles of muscle during different maturity stages

IMMATURE - MUSCLE

CHROMATOGRAM 9 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 320

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.435	6091			100	
	TOTAL	6091			100	

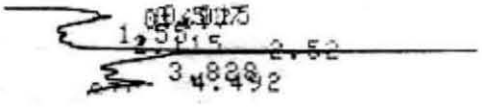
MATURE - MUSCLE

CHROMATOGRAM 13 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 324

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.523	4493			100	
	TOTAL	4493			100	

SPENT - MUSCLE



CHROMATOGRAM 16 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 327

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.315	2015	V		12.3904	
2	0.697	1813	V		11.1511	
3	1.55	1047	V		6.4364	
4	2.52	7827	V		48.1337	
5	3.828	1492	V		9.172	
6	4.492	2068	V		12.7165	
	TOTAL	16262			100	

carotenoid concentration of 300 to 400 µg/g in mature ovaries of *Penaeus schmitti.* Higher values are recorded in *P. homarus* when compared with the concentration in ovaries of *P. schmitti,* but these results are comparable with the results obtained in the ovaries of deep water shrimps where concentration was 400 µg/g to 600 µg/g (Herring, 1973).

Carotenoid concentration in the ovary of *P. homarus* was lower in the immature stage and increased to the highest value in the mature stage. Spent stage ovary showed a decline in carotenoid concentration when compared to that of the mature ovary. The changes in the carotenoid concentration in the ovaries were apparent from the changes in the colour pattern of the ovaries. The immature ovary was white in colour, and as the ovary matured the colour changed to brick red. The caroteniod level in the immature ovary was 0.01% and as the ovary matured the concentration became eight fold. This apparent change in caroteniod level resulted in a significant change in the colour pattern of the ovary. In the spent ovary there was a marked reduction in colour and the ovary had a beige colour. The marked changes in colour and concentration of carotenoids in the ovary during different maturity stages showed that mobilization of carotenoids occur in *P. homarus.*

Sexual maturation in salmonids causes mobilization of carotenoids from storage organs and their selective transfer to the skin and gonads (Crozier, 1970; Sivtseva and Dubrovin, 1980; Kittahara, 1983; Bjerkeng, 1992; Hatlen *et al,* 1997). During ovarian maturation, vitellogenesis causes depletion in the carotenoid content of the hepatopancreas (Castillo *et al.,* 1982). Hepatopancreas acts as the carotenoid storage organ in crustaceans. The

pattern of mobilization was from the hepatopancreas to exoskeleton and reproductive organs and was routed through the glycoprotein intermediates via the haemolymph. The exoskeletal carotenoid concentration in the immature stage was lower when compared with that of the mature stage. During the spent stages a declining trend in exoskeletal carotenoid concentration was observed. This could be because of the utilization of carotenoid reserves for spawning purpose. The reduction in total carotenoid concentration in the hepatopancreas of mature *P. homarus* and the concomitant increase in ovary and exoskeleton points to the role of hepatopancreas in mobilizing these pigments during ovarian maturation.

The non-detection of canthaxanthin in ovary and other tissues clearly indicates that the dietary carotenoids are modified in *P. homarus* and astaxanthin is the main carotenoid in the species. However, the presence of minor peaks in mature and spent ovary, especially in maturation indicates that other carotenoids may also have some role during maturation.

Muscle pigment concentration in mature salmonids was found to be lower than those of immature fishes. (Crozier, 1970; Kittahara, 1983; Torrisen and Naevdal,1984; Torrisen, 1985; Ando and Hatano, 1987; Bjerkeng,1992). This was mostly due to the redistribution to skin and gonads in mature females. A similar phenomenon was found in mature *P. homarus*. The immature lobsters had a higher concentration of muscle carotenoids which declined as the lobsters matured. This decrease could be attributed to the mobilization of carotenoids to exoskeleton and gonads. The carotenoid derived skin colour of female salmonids is considered to represent a secondary sexual characteristic

having a functional role in courtship and breeding behaviour. Mobilization of carotenoids to the skin of many fishes for its breeding attire has been well documented. Such breeding colouration is not present in spiny lobsters. Yet the deposition of carotenoids in exoskeleton at maturity is as interesting as the reduction after spawning. The carotenoid reserves deposited in the exoskeleton in *P.homarus* at maturity may be mobilized for final deposition in the ova or for the spawning process.

A decrease in the astaxanthin content in the hepatopancreas occurs as the lobster matures. This decrease in astaxanthin content coincides with increase in astaxanthin concentration of exoskeleton and ovary. This suggests the mobilization of astaxanthin from the hepatopancreas to the ovary and exoskeleton. The mobilization and subsequent redistribution to the gonads confirm the reproductive function of carotenoids in general and astaxanthin in particular.

During early maturation, astaxanthin accumulates in the hepatopancreas. During secondary vitellogenesis they are mobilized from the hepatopancreas to the ovaries (Vincent *et al.*,1998). Dall *et al.* (1995) reported astaxanthin as the principal carotenoid in the ovaries of maturing females of *Penaeus esculentus*. Astaxanthin levels in the ovaries of *P. esculentus* rose from 2 to 34 ppm and in the hepatopancreas the increase was from 20 to 120ppm. In the exoskeleton of *P. esculentus* the astaxanthin content remained relatively constant. The astaxanthin content in the ovaries of *P. homarus* almost doubled as the lobster matured which is in accordance with the findings of Dall *et al.*, (1995). In contrast to the increasing trend of astaxanthin in the hepatopancreas of

P. esculentus, a decreasing pattern has been observed in the astaxanthin content of hepatopancreas of *P. homarus* as the lobster matured. In the hepatopancreas of mature *P. homarus* a decrease of nearly 120 µg/g of astaxanthin was reported. This could be due to mobilization to the ovaries. Contrary to the studies in *P. esculentus* the astaxanthin level in the exoskeleton in *P. homarus* varied through out the maturation cycle. The highest level was in the mature stage, and lowest in the spent stage. The decrease in the astaxanthin level in the muscle of the mature lobster could be due to mobilization and the increase in astaxanthin level in the spent stage can be due to resorption of the pigment from the ovary.

To elaborate further on the reproductive function ascribed to astaxanthin, it can be assumed that this pigment can influence the egg quality and thereby the hatching rate and larval survival. The effect on larval survival can be attributed to the anti-oxidant properties of astaxanthin. Free radicals initiated by various factors including active oxygen attack lipids and proteins in biomembranes leading to deterioration of egg quality. Since astaxanthin acts as strong scavengers of free radicals (Graham,1989;Henk,1999) they can protect eggs from oxidative deterioration, thus improving larval survival. Astaxanthin can play an important role in providing the necessary reserves in embryos and prefeeding larval stages for development of chromatophores.

To get a clear picture of mobilization of nutrients to different tissues during maturation, the values have to be quantified for expressing the changes in concentration alone (Vijayakumaran,1990). The values of carotenoids in different tissues of *P.homarus* during ovarian maturation in a uniform weight of

hundred gram body weight as in table 9 and fig.5. show in increase of 1.35mg in mature ovary and 7.2mg in the exoskeleton while the corresponding reduction in hepatopancreas and muscle are 0.91mg and 1.05mg. This clearly indicates that the carotenoids are continuosly routed to the ovary and exoskeleton through the hepatopancreas and muscle. Spawning results in reduction of carotenoids in ovary (1.41mg) and exoskeleton (12.82mg) and moderate resorption (1.48mg) in the muscle. The drastic reduction of 12.82mg carotenoid from exoskeleton during spawning requires further investigation.

6. EFFECT OF CAROTENOIDS IN NATURAL DIETS ON PIGMENTATION PATTERN IN *PANULIRUS HOMARUS*

Carotenoids are the pigments that impart a myriad of colours in aquatic animals. In salmonids like trout, salmon and charr they impart a red colour to the flesh and in crustaceans these polyene pigments contribute to the colouration of exoskeleton and flesh. It has been conclusively established that fish do not posses the ability to synthesise carotenoids. Crustaceans also are unable to synthesize carotenoids. Hence, the pigmentation pattern of fishes and crustaceans results from the pigments absorbed from the diet and accumulated in the tissues (Steven,1949; Goodwin,1951; Fox,1957; Hata and Hata,1973; Foss *et al.*,1984; Torrisen,1986; Storebakken, *et al.*,1986). The changes in the amount and redistribution of carotenoid pigments in crustaceans are dependent on several factors including embryogenesis, sexual cycle, moulting, food, and habitat. Food and habitat are the extrinsic factors contributing to the pigmentation pattern. The duration of feeding, the carotenoid concentration in the diet and the source of carotenoid are the major factors which determine the level of pigmentation in various tissues and organs in crustaceans.

Lobsters are selective about the food they eat, despite being known as scavengers or omnivores (Radhakrishnan, 1989). Gut content analysis reveals the feeding habits and feeding preferences of organisms in their natural habitat. Food and feeding preferences of lobsters as revealed through gut content analysis shows that lobsters feed mainly on crustaceans, molluscs, and fishes

with preference for the former two over the latter. Diet of *Panulirus homarus* was dominated mainly by mussels, (Berry, 1971) and that of *Homarus americanus* by crabs.

Crustaceans deposit the ingested carotenoids in the exoskeleton and muscles. In fishes and shrimps which accept pelleted feeds, pigmentation studies using synthetic dietary carotenoids are easier. Generally shrimp are fed starter, grower and finisher feeds. The starter and grower feeds contain higher levels of protein and are used throughout the grow out phase. The finisher feeds are generally given towards the end of the grow out period just prior to harvest. In addition to other nutrients, these finisher feeds contain carotenoids that impart necessary colour to cultured shrimps. No commercial diet has yet been formulated for spiny lobsters, though few pelleted diets have been found acceptable to *P. homarus*. The pelleted feeds could not produce significant growth since the animal tends to spend more energy on feeding and the feed loss is very high. The heavy feed loss deteriorates the water quality and causes high risk of mortality (Radhakrishnan and Vijayakumaran, 2000). Earlier studies have shown that lobsters have a preference for fresh feeds over the pelleted feeds (Radhakrishnan, 1989; Vijayakumaran, 1990; Radhakrishnan and Vijayakumaran, 2000). The preference for fresh feeds and non-availability of commercial pelleted feeds are the main reasons for using live clams and mussels as food for lobsters in fattening practices.

Studies concerning the carotenoid composition of food items of lobsters and their contribution to pigmentation pattern are meagre. The present study

was undertaken to evaluate the carotenoid profile of natural food items like green mussel and prawn and also the alternative food items like clams which are used for fattening purposes, and their role in imparting natural colour to *P.homarus* . Visual judgement of the colour pattern of lobsters fed on different diets was also carried out which were supported by photographic evidence.

6.1. RESULTS

6.1.1. Carotenoid concentration of different feeds

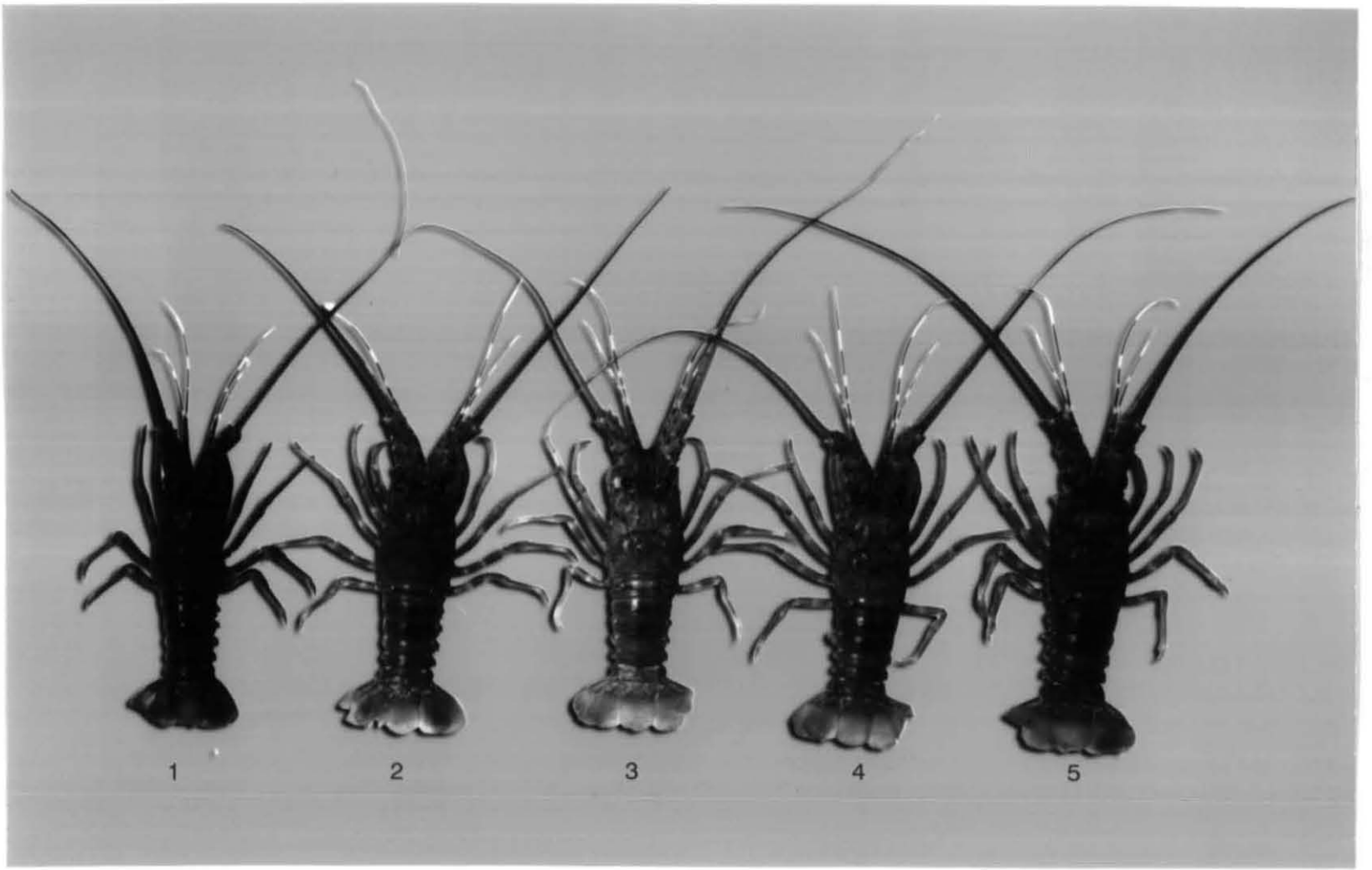
The four feeds chosen for the study were the bivalves *Donax cuneatus, Perna viridis, Paphia malabarica* and the penaeid prawn *Metapenaeus dobsoni.* The carotenoid concentration of the four feeds was estimated. *Donax cuneatus* had a carotenoid concentration of 240µg/g. The carotenoid concentration of *Perna viridis, Paphia malabarica* and *Metapenaeus dobsoni* were 500µg/g, 356µg/g, and 645µg/g respectively. The qualitative estimation of carotenoids was also carried out. *D.cuneatus* showed astaxanthin concentration of 128µg/g, whereas *P.viridis* registered 346µg/g. *P. malabarica* contained 105µg/g of astaxanthin while *M. dobsoni* had 387µg/g (Table 13) (Fig 10,11).

6.1.2. Visual judgement scores

There was apparent colour differences in the exoskeleton of lobsters fed on different diets as is evident from the photograph (plate 1). Visual judgement of the colour of exoskeleton of lobsters fed on different diets showed the following pattern; Group D fed on *M. dobsoni* had a bright colour (brownish red colour). The colour pattern of group B fed on *P. viridis* was almost

1. Lobster caught from the wild
2. Group B - fed on *Perna viridis*
3. Group A - fed on *Donax cuneatus*
4. Group C - fed on *Paphia malabarica*
5. Group D - fed on *Metapenaeus dobsoni*

Plate 1. Colour pattern of *Panulirus homarus* fed on natural diets.



6 cms

TABLE 12. Carotenoid concentration (μg/g) of different feeds

Feeds	Total Carotenoid (mean±SD)	Astaxanthin *
Donax cuneatus	240 ± 24	128
Perna viridis	500 ± 47	346
Paphia malabarica	356 ± 29	105
Metapenaeus dobsoni	645 ± 61	387

* Standard deviation was not given since the samples were pooled.

TABLE 13. Carotenoid concentration (μg/g) of different tissues of *P. homarus* reared on different feeds

TISSUES	Group A *	Group B **	Group C ***	Group D ****
	(Mean ± SD). n = 3			
Hepatopancreas	82 ± 7	233 ± 20	177 ± 25	263 ± 15
Exoskeleton	185 ± 7	537 ± 15	303 ± 15	547 ± 9
Muscle	34 ± 3	45 ± 5	37 ± 2	54 ± 2

* Group A - fed on *Donax cuneatus*
* * Group B - fed on *Perna viridis*
*** Group C - fed on *Paphia malabarica*
**** Group D - fed on *Metapenaeus dobsoni*

TABLE 14. ANOVA FOR DIFFERENT FEEDS

HEPATOPANCREAS

Source of Variation	SS	df	MS	F
Between Groups	57275.7	3	19091.9	56.5
Within Groups	2704	8	338	
Total	59979.7	11		

EXOSKELETON

Source of Variation	SS	df	MS	F
Between Groups	287089	3	95696.3	16.68
Within Groups	45900.7	8	5737.6	
Total	332989.7	11		

MUSCLE

Source of Variation	SS	df	MS	F
Between Groups	758	3	252.7	25.7
Within Groups	78.7	8	9.9	
Total	836.7	11		

identical to that of group D. The colour pattern tended to fade in group C fed on *P. malabarica* when compared with that of the former two. Group A fed on *D.cuneatus* had a pale colour and was the least pigmented among the four groups when judged visually.

6.1.3. Carotenoid concentration in hepatopancreas

The carotenoid concentration of hepatopancreas of group A fed on *D. cuneatus* was 82µg/g, while those of group B, C and D fed on *P. viridis, P.malabarica* and *M. dobsoni* respectively were 233µg/g, 177µg/g and 263µg/g in that order. The results of Duncans multiple range test showed a comparison of carotenoid concentration in the hepatopancreas of lobsters receiving four different diets. (Appendix III). There was significant difference between all the groups except for group B and group D.

6.1.4. Carotenoid concentration in the exoskeleton

Carotenoid levels in the exoskeleton of lobsters receiving different diets showed significant difference. Exoskeleton of group A fed on *D.cuneatus* had a concentration of 185µg/g. Group B fed on *P.viridis* had a concentration of 537µg/g, while the exoskeleton of group C and D fed on *P.malabarica* and *M.dobsoni* respectively had a concentration of 303µg/g and 547µg/g in that order. Groupwise comparison of carotenoid concentration in the exoskeleton by Duncans multiple range test showed significant variation between all the groups with the exception of group B and group D. There was little difference between group B and group D. The results showed that among the four groups, group B

Fig.10. Chromatographs showing carotenoid profiles of different feeds

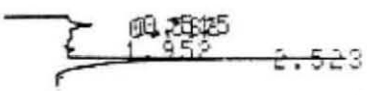
PAPHIA MALABARICA

CHROMATOGRAM 4 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 315

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.42	16757			86.5695	
2	3.33	2600	V		13.4305	
	TOTAL	19357			100	

DONAX CUNEATUS



CHROMATOGRAM 14 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 325

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.523	2614	V		100	
	TOTAL	2614			100	

Fig.11. Chromatographs showing carotenoid profiles of different feeds

PERNA VIRIDIS

CHROMATOGRAM 2 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 313

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.425	23929	S		100	
	TOTAL	23929			100	

METAPENAEUS DOBSONI

CHROMATOGRAM 3 MEMORIZED

CHROMATOPAC C – R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 314

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.575	1300	V		4.6646	
1	0.683	3411	V		11.5309	
1	2.428	24793	V		83.8045	
	TOTAL	29584			10	

and group D fed on *P. viridis* and *M. dobsoni* were better than group A and group C fed on *D. cuneatus* and *P. malabarica* respectively.

6.1.5. Carotenoid concentration in the muscle

Muscle carotenoid concentration of group A fed on *D. cuneatus* was the lowest (34µg/g). The muscle carotenoid concentration was 45µg/g in group B fed on *P viridis*. Group C fed on *P.malabarica* showed carotenoid concentration 37µg/g while Group D fed on *M. dobsoni* had the highest concentration of 54µg/g. Groupwise comparison of carotenoid concentration showed significant variation between all the groups with the exception of group A and group C.

6.2. DISCUSSION

In shellfishes, particularly the decapods which are marketed whole (shell on), the colour of the exoskeleton is an important criterion for marketing. The Indian spiny lobster *P. homarus* is one of the highly priced seafood, which is marketed live. The colour of the species in the wild is greenish brown and the colour fades when they are being held in captivity for fattening purposes. Colourless appearance of cultured decapods have been reported by several authors (Lenel ,1961; Petit *et al.*,1998). The pale appearance in the lobsters reared in captivity aroused an interest for analyzing the carotenoid content of lobster fed on different diets.

The degree of pigmentation of exoskeleton in lobsters can be characterized in two ways: by chemically analyzing the quantitative carotenoid

of the exoskeleton or by the definition of colour appearance. Definition of colour appearance can be done by subjective colour perception or by the characterization of colour by measuring its composition instrumentally. The results of chemical analysis were in accord with those obtained by visual judgement. Group D and Group B which were fed on *M.dobsoni* and *P.viridis* respectively had the brightest colour and their carotenoid concentrations were the highest when compared with other groups. Group A which had the lowest carotenoid concentration, was the least pigmented among the four groups. Among the few studies concerning the role of natural feed on the pigmentation pattern in lobsters, studies on eye-stalk abalated *P. homarus,* which were fed with green mussel, brackishwater clam, trash fish and a combination of all the three showed that the lobsters fed with mussel and mixed food retained natural colouration. (Vijayakumaran and Radhakrishnan, 1984; Radhakrishnan, 1989). The colour of lobsters fed on clam and fish became pale producing even 'albino' lobsters. An interesting observation was that these albino lobsters were able to regain their natural colour to a certain extent when they were fed with green mussel (Vijayakumaran, personal communication).

The relative proportion of carotenoids fluctuates according to the species. Variations in the pigment content are also dependent on numerous physiological and ecological prarmeters. Basically, carotenoid levels reflect the availability of pigment in the food and the appetite of the animal for it (Castillo *et al.*,1982). Correlating the carotenoid and astaxanthin levels in the feed on the pigmentation pattern, it is found that the carotenoid level in the feed has a direct relationship with pigmentation pattern of the lobster. The carotenoid content is species specific (Goodwin, 1984; March and Macmillan, 1996). Mussel and

prawn had higher levels of carotenoids when compared to clams. During the experimental period the preferences of lobsters were for mussels and shrimps when compared to clams. The appetite for these feeds together with the higher levels of carotenoids accounted for better pigmentation pattern in lobsters receiving these two diets. Differences in absorption and tissue deposition of pigments in *P.homarus* is well documented in the present study. The high similarity of the carotenoid in the diet to those present in the lobster could be another cause for better pigmentation pattern. The low level of pigmentation in the clams could be due to the poor absorption rate from its feed. Mussels have high glycogen content and this could have facilitated better movement of carotenoids from gut to the hepatopancreas as mobilization of carotenoids occur via the haemolymph through glycoprotein intermediates (Castillo *et al.*, 1982). The brightest colour observed in group D fed on crustacean carotenoid was due to the high level of astaxanthin as astaxanthin is reported to be the major pigment responsible for the colour of exoskeleton. Crustacean sources of carotenoids were found to enhance pigmentation in salmonids (Tanaka *et al.*,1976; Spinelli and Mahnken 1978; Simpson and Kamata,1979). Similar results were obtained in *P.homarus* fed with prawn. The carotenoid pigments from the diet are absorbed directly from the food and passed across gut walls into the body fluids to be distributed to other tissues and organs. The transfer of carotenoids from the gut to the hepatopancreas is accomplished through 'slow glycoprotein intermediates' (Castillo *et al.*,1982).

The colour of the hepatopancreas of lobsters fed with different diets had a direct relationship to the colour of pigment extracts from the diet. The pigment extracts of razor clam had a pale green tinge, while the extracts of green mussel had a brown colour. The extracts of textile clam was yellowish orange

and that of prawn was orange in colour. The colour pattern was reflected in the hepatopancreas when the animals were sacrificed at the end of feeding experiments. This illustrates the fact that the carotenoids of the diet has a direct influence in controlling the pigmentation pattern (Goodwin, 1984; March and Macmillan, 1996). This also supports the fact that hepatopancreas acts as the central organ for biotransformation of carotenoids (Segner *et al.*,1989). The movement from the hepatopancreas to the exoskeleton occurs via haemolymph through glycoprotein intermediates. The pigmentation of the exoskeleton is dependent on the rate of mobilization from the hepatopancreas. The low rate of deposition in the hepatopancreas or the slow mobilization from hepatopancreas can also result in the low levels of pigmentation. In general it can be concluded that besides the nature and amount of dietary carotenoids pigments, the rate of deposition in the hepatopancreas and the mobilization from the hepatopancreas are major factors controlling pigmentation pattern.

The carotenoid profile of the animal changes according to the physiological status of the animal. As revealed in the studies in the previous chapters, maturation and moulting are important physiological changes that can influence carotenoid concentration and also the pigmentation pattern of the animal. The results of the present study suggest that the choice of the right stage be it in the moult cycle or in the maturation cycle coupled with the provision of the best feed in terms of its nutritive value and carotenoid concentration will aid in achieving the best pigmentation pattern. Normally clams are provided as food for fattening purposes. Results of the present study indicate that mussels and prawns are superior to clams in imparting better colour pattern in spiny lobster *P. homarus.*

7. EFFECT OF CAROTENOID ENRICHED NATURAL DIET ON PIGMENTATION OF *PANULIRUS HOMARUS*

Carotenoids form complexes with proteins. These carotenoproteins occurring in the chromatophores are responsible for the colour of the exoskeleton in shellfishes like shrimps and lobsters. Astaxanthin was the major carotenoid pigment in shrimp *Pandalus borealis* and *Penaeus japonicus* (Katayama *et al.*,1972) whereas, *Metapenaeus affinis* contained lutein and ß carotene. Carotenoid analysis of carapace of the crab *Portunus trituberculatus* revealed the presence of astaxanthin, canthaxanthin and echineone. These polyene pigments occurring as carotenoprotein complexes undergo denaturation when boiled and is responsible for the change of colour in exoskeleton in shellfishes.

Crustaceans have the ability to transform the pigments of their diets to endogenous forms. The dietary carotenoids are absorbed and after a series of metabolic transformations are deposited in the exoskeleton. The colour of finfish and shellfish are more attractive when they are caught from the wild. When held under captive conditions the natural colouration tends to fade (Tomiyama, 1974; Menasveta *et al.*, 1993). The loss of colour can be attributed to the total change in the rearing conditions and also to the effect of food. Variations in carotenoid deposition in crustaceans occur as a result of changes in ecological parameters. Studies have revealed that the natural greenish brown colour in lobsters tend to fade when they are held in captive conditions for fattening purposes (Radhakrishnan and Vijayakumaran, 2000). Lobsters fed on clams became pale in colour. Since the lobsters are marketed alive any change in

colour is viewed negatively by the consumers during quality evaluation and affects the acceptability as well as the marketability of the product. Fortification of the diets with carotenoids was the only alternative to improve the colouration of the product. In finfishes and shellfishes like shrimp which accept formulated diets, supplementation with carotenoids is easier. Since lobsters do not accept formulated diets, enrichment of natural feeds seems to be the only alternative. So in the present study lobsters were fed on clams enriched with two algal sources of astaxanthin namely, *Spirulina* and *Haematococcus pluvialis*.

7.1. RESULTS

7.1.1. Visual judgement scores

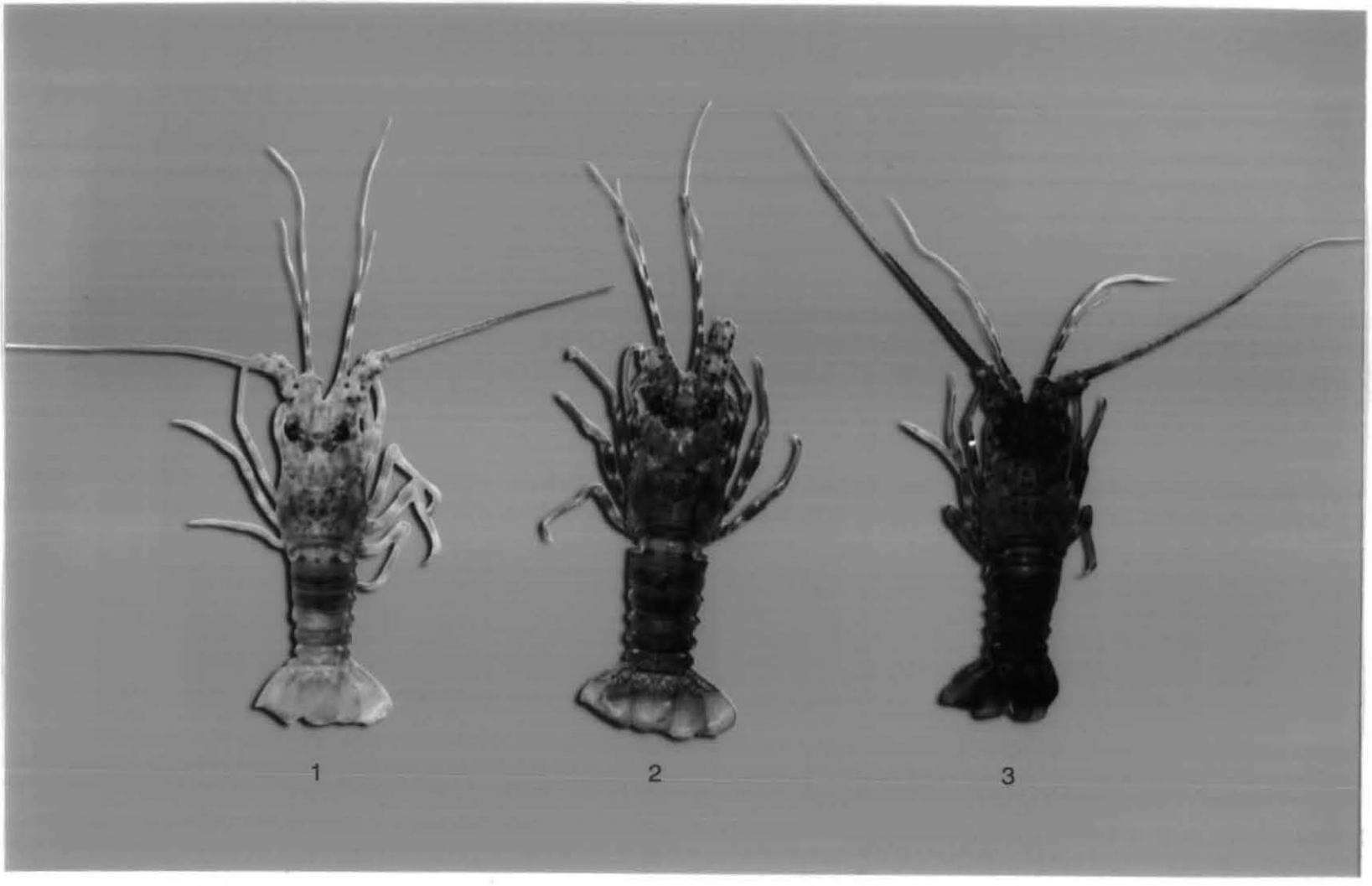
The lobsters fed on live clam *D. cuneatus* served as control and was pale in colour. The colour pattern of group A fed on clams enriched with *H. pluvialis* were darker when compared with control. Group B lobsters fed on clams enriched with *Spirulina* had the darkest hue when compared with control and with group A (Plate 2).

7.1.2 Enrichment Studies

Carotenoid analysis in *H. pluvialis* and *Spirulina* showed that the former had 200µg\g and the latter contained 160µg/g. Enrichment studies were done in *D. cuneatus* which had the lowest carotenoid concentration among the four feeds analysed (Table 12). To determine the ideal algal concentration for enrichment, the clams were exposed to 4 algal concentrations for 1 hour. The concentrations studied were 0.5mg/ml, 1mg/ml, 1.5mg/ml and 2mg/ml. The algal concentrations of 0.5mg/ml and 1mg/ml did not improve the carotenoid concentration in the clams. The clams exposed to algal concentrations of

1. Control group - fed on *Donax cuneatus* without enrichment
2. Group A - fed on *Donax cuneatus* enriched with *Haemato coccus pluvialis*
3. Group B - fed on *Donax cuneatus* enriched with *Spirulina*

Plate 2. Colour pattern of *Panulirus homarus* fed on carotenoid enriched natural diets.



15 cms

TABLE 15 a. Carotenoid concentration of algae

Algae	Cell Size	Appearance	Family	Carotenoid Concentration
Spirulina	30 – 40 μ	Green	Chlorophyceae	169μg/g
Haematococcus pluvialis	40 – 50 μ	Red	Chlorophyceae	200μg/g

TABLE 15 b. Carotenoid Concentration of *D.cuneatus* enriched with *Spirulina* at 2mg/ml at 1 hour intervals.

0 Hour	1 Hour	2 Hour	3 Hour	4 Hour	5 Hour	6 Hour
239	280	280	272	262	250	248
235	280	279	275	263	255	242
238	279	279	274	260	259	245
240	275	280	270	264	254	248
234	276	275	270	263	252	249

TABLE 15 c. Carotenoid Concentration of *D.cuneatus* enriched with *Spirulina* at different concentrations.
(Carotenoid concentration expressed as μg/g)

Clams without enrichment	Clams enriched with algae at 0.5 mg/ml	Clams enriched with algae at 1 mg/ml	Clams enriched with algae at 1.5 mg/ml	Clams enriched with algae at 2 mg/ml
239	240	255	268	278
235	236	256	270	279
238	238	258	265	278
240	239	259	269	270
234	235	260	265	269

TABLE 16. Carotenoid concentration (μg/g) of different tissues of *P. homarus* fed carotenoid enriched diets

Tissues	Control Group *	Group A **	Group B ***
	(Mean ± SD). n=3		
Hepatopancreas	79 ± 3	155 ± 3	226 ± 2
Exoskeleton	181 ± 9	296 ± 2	513 ± 42
Muscle	33 ± 3	41 ± 2	59 ± 7

* Control group - fed on *Donax cuneatus* without enrichment
* * Group A - fed on *Donax cuneatus* enriched with *Haemato coccus*
*** Group B - fed on *Donax cuneatus* enriched with *Spirulina*

TABLE 17. ANOVA FOR ENRICHED FEEDS

HEPATOPANCREAS

Source of Variation	SS	df	MS	F
Between Groups	32574.89	2	16287.44	1832.3
Within Groups	53.3	6	8.9	
Total	32628.2	8		

EXOSKELETON

Source of Variation	SS	df	MS	F
Between Groups	170402.9	2	85201.4	137.1
Within Groups	3728.7	6	621.4	
Total	174131.6	8		

MUSCLE

Source of Variation	SS	df	MS	F
Between Groups	1070.9	2	535.4	20
Within Groups	160.7	6	26.8	
Total	1231.6	8		

2mg/ml showed an increase of nearly 40µg/g (Table 15c). The ideal time period for enrichment was determined by exposing the clams to filtered seawater containing algae at a concentration of 2mg/ml for six hours. Samples were analysed for carotenoids after every 1 hour. Carotenoid analysis, after exposing the clams for 1 hour and 2 hours showed that enrichment occurred. From the third hour to sixth hour the carotenoid concentration was decreasing showing that enrichment did not occur. At the sixth hour the carotenoid concentration of the clams was almost equal to those that were not enriched. Carotenoid concentration of clams enriched for 1 hour and 2 hours were almost same (Table 15b). Hence 1 hour was found to be the ideal time duration for enrichment.

7.1.3. Carotenoid concentration of hepatopancreas

Significant changes in carotenoid concentration in the hepatopancreas were recorded among the groups ($P<0.05$) (Table 17). Group wise comparison of carotenoid concentration in the hepatopancreas using Duncans multiple range test showed that there was significant difference among the groups (Appendix IV). The carotenoid concentration of hepatopancreas of control group fed on clams without enrichment had a carotenoid concentration of 79µg/g (Table 16). The carotenoid concentration increased to 155µg/g in group A fed with clams enriched with *H. pluvialis* and a further increase to 226µg/g was seen in group B fed on clams enriched with *Spirulina*. Comparison of group A and group B with the control group using Duncans multiple range test revealed that group B fed on clams enriched with *Spirulina* was superior to group A fed on clams enriched with *H. pluvialis*.

7.1.4. Carotenoid concentration of exoskeleton.

Statistical analysis of carotenoid concentration of exoskeleton showed significant variations ($P<0.05$). The control group fed on clams without enrichment had a carotenoid concentration of 181µg/g. The carotenoid concentration group A fed on clams enriched with *Haemotococcus* was 296 µg/g, while the carotenoid concentration of group B fed on *Spirulina* enriched clam was 513 µg/g. As in the case of hepatopancreas comparison of carotenoid concentration in the exoskeleton using Duncans multiple range test showed that group B fed on clams enriched with *Spirulina* was the best followed by group A fed on clams enriched with *H. pluvialis.*

7.1.5. Carotenoid concentration of muscle

Carotenoid concentration of muscle showed significant variation ($P<0.05$). Muscle carotenoid concentration was 33 µg/g in the control group which showed a progressive increase in group A fed on the clams enriched with *H. pluvialis* (41µg/g) and group B fed on the clams enriched with *Spirulina* had a concentration of 59µg/g. Results of Duncans multiple range test showed that carotenoid concentration in the muscle of group B was significantly different from group A and control group. There was little difference between group A and control group.

7.2. DISCUSSION

The colour of exoskeleton in one of the sensory attributes that gains a lot of importance in quality evaluation of crustaceans. It is of great economic importance to achieve a natural pigmentation in farmed animals. Since crustaceans are not able to synthesise astaxanthin, it has to be supplemented

in the diet. Various authors have shown that fish can be pigmented by inclusion in the diet of astaxanthin sources such as crustaceans, crustacean by-products and pigment extracts of these (Saito and Reiger,1971; Spinelli and Manhken,1978; Torrisen,1982). Lobsters fed on the green mussel have been reported to regain natural colouration. The colour patterns developed are different for the different pigment sources. The rate of colour manifestation can depend on the amount and nature of the carotenoid pigments in each pigment source. The variations in pigmentation can also result from differences in the rate at which astaxanthin is mobilized.

The pigmentation pattern of the lobsters fed on enriched diets were better than those fed on the clams without enrichment. The carotenoid enriched diets were superior to control group in terms of carotenoid concentration. It is seen that the addition of pigmentation agent to the food can intensify either the visual impression of colour, or the pigment concentration in products of animal origin. It is important to note that one of these effects does not necessarily imply the other (Bauernfeind *et al.*, 1971; Brubacher,1972). The metabolic pathway from betacarotene to astaxanthin in *Panulirus japonicus* occurs according to the following pattern. ß carotene → isocryptoxanthin → echinenone → 4' hydroxy-echinenone → canthaxanthin → 3- hydroxy canthaxanthin → astaxanthin. The conversion from ß carotene to echineone occurs in the hepatopancreas and formation of astaxanthin and other intermediate products occur in the cells of other organs like exoskeleton (Katayama *et al.*,1973).

The qualitative analysis of carotenoid of *H. pluvialis* showed the presence of ß carotene, astaxanthin, canthaxanthin and lutein (Choubert and Heinrich,1993), while those of *Spirulina* contained ß carotene, echineone, cryptoxanthin, xeaxanthin and myxoxanthophyll (Choubert,1979). The

carotenoid intermediates of *H. pluvialis* was almost similar to those present in the lobster. *Spirulina* enriched diets gave a better pigmentation pattern when judged visually and this was supported by the higher carotenoid level in the exoskeleton. It has been reported that in crustaceans the carotenoid level in the feed has a direct relationship on the pigmentation pattern (Castillo *et al.*,1982). The total carotenoids as well as the percentages of astaxanthin was high in *H. pluvialis* when compared with those in *Spirulina* . *H. pluvialis* when subjected to stressed cultural conditions becomes red in colour due to the formation of large amounts of carotenoids (Goodwin, 1971). ß- carotene and its keto derivatives are found outside the chloroplast in the aplanospores of *H. pluvialis* (Czyan and Kessler,1967). The final carotenoid concentration in the exoskeleton of lobsters fed on clams enriched with *H. pluvialis* was 296µg\g and those fed on *Spirulina* enriched diets was 513µg\g. Despite higher levels of carotenoids in *H. pluvialis,* the pigmentation pattern in the exoskeleton of lobsters fed *H. pluvialis* enriched clams was poor. The carotenoid concentration of H. *pluvialis* enriched diets was low. A possible limiting factor is the availability of carotenoid from the algae. Highly pigmented algae indeed occur in an encysted form surrounded by a thick cell wall and this barrier may impede the absorption of pigments (Johnson and An, 1991) and this would have resulted in the low pigmentation pattern of lobsters fed on *H. pluvialis* enriched diets.

Pigmentation pattern in clawed lobsters suggests that the carotenoid provision via live feeds gave more efficient results (D'Abramo et al., 1983). The rate of uptake of carotenoids in clams could be another cause for the lower pigmentation pattern of lobsters fed on *H. pluvialis* enriched clams. The filteration rate of clams provided with *Spirulina* was much higher that that of *H. pluvialis*. This could be another reason for the better pigmentation pattern

found in the group fed on *Spirulina* enriched diets. Clams being filter feeders can take in a lot of algae without actually retaining them in their tissues resulting in the production of pseudofaeces. This glutton effect and production of pseudofaeces could have occurred in clams fed on *H. pluvialis* resulting in lower accumulation in the tissues and lower pigmentation rate in target organisms like lobsters.

During fattening process, normally clams are provided as feed for lobsters. Studies have shown that lobsters fed on clams became pale and that the colour can be regained by feeding them with mussel (Vijayakumaran and Radhakrishnan,1984).The present study on naturally occurring dietary carotenoids (chapter 6) has shown that lobsters fed on clams had the lowest rate of carotenoid accumulation in the exoskeleton. The rate of deposition of carotenoids in the exoskeleton of lobsters fed on mussels and prawns was high.

Mussels and prawns are found to be superior to clams for fattening purposes in terms of carotenoid analysis. Clams are utilized as feed because of their availability and cheaper price, in comparison to other food items. Therefore ways to improve the carotenoid content of clams would be of use in lobster fattening. Results of the present study indicated that lobsters fed on clams enriched with *Spirulina* had almost similar levels of carotenoids as those fed with mussels and prawns.

Mass culture of *Spirulina* can be done and for fattening purposes clams can be enriched by feeding with this alga. Another alternative would be a polyculture system where in *Spirulina,* clams and lobsters can be reared in the same tank during fattening period.

8. INFLUENCE OF THE COLOUR OF REARING TANKS ON PIGMENTATION OF *PANULIRUS HOMARUS.*

Body colouration in Crustacea with strong calcified exoskeleton is essentially that of the pigmented layer. Though the basic chromatic pattern of crustaceans with highly calcified cuticle are genetically determined, they can vary with environmental changes. Colour change in crustaceans consists of relatively slow morphological and physiological changes. Morphological changes arise from quantitative alterations in the epidermal pigments whereas physiological colour changes are caused by migrations of pigment granules or of the entire chromatophores. Pigmentary effectors or chromatophores are specialised cells that are able to alter the quantity and the display of the pigments contained in them, and thereby contributing to the adaptive colour changes. Depending on the colour of the pigment contained in the chromatophores they can be melanophores (black / brown) leucophores (white), erythrophores (red) and xanthophores (yellow). The total colouration of crutaceans is determined by the number, type and distribution of chromatophores. Quantitative changes in any of the components of pigmentary system can lead to the slow transformation in colouration. These alterations causing morphological colour changes or chromogenic changes may occur in response to the relevant environmental conditions, and also in association with development, growth and sexual maturity (Knowles and Callan,1940; Crane, 1975).

Morphological colour changes involving alterations in the number of chromatophores have been reported in many species of crustaceans including *Procambarus clarkii* (Bowman, 1942) *Ocypode ceratopthalmus* (Green, 1964)

Athanas niteseens, *Crangon crangon*, *Hippolyte varians*, and *Palaemon elegans* (Chassard - Bouchard, 1965). Besides alterations in the number of chromatophores, alterations in the quantity and type of pigments within a cell, are known to contribute to the morphological colour changes (Brown, 1934; Robinson and Charlton, 1973).

Physiological colour changes are displayed not only as an adaptation to match the background colouration but also in response to a wide range of stimuli, including transient fluctations in illumination and temperature. During these adaptations, chromatophore pigment movements are elicited by a reflex mechanism involving the eyes and the neuroendocrine system, which are secondary responses. Primary responses occuring through extraocular routes also contribute to the adaptations to the background colours (Nagabushanam, 1965).

Chromatophore responses to background colour change is influenced by several factors. Among these factors are light intensity, temperature, circardian rhythms (Brown and Sandeen, 1948; Fingerman, 1956), longterm adaptation to specific backgrounds (Fingerman, 1957), geographical area from which the specimens are collected, and also the stages in larval development and metamorphosis (Keeble and Gamble, 1904).

The present study was undertaken to evaluate the effect of different background colours on the pigmentation pattern of the Indian spiny lobster *P. homarus* under captive conditions, as the colour of the rearing tank might influence pigmentation in the spiny lobster *P.homarus*. The lobsters were fed on the clams *D. cuneatus* during the period of study.

8.1. RESULTS

8.1.1. Carotenoid concentration of hepatopancreas

Significant changes were recorded in the hepatopancreas of lobsters reared in different coloured tanks as per anova and Duncans multiple range test ($P<0.05$) (Table 19). Groupwise comparison showed that there was significant difference among the groups (Appendix V). The hepatopancreas of lobsters reared in white coloured tank had the lowest carotenoid concentration of 45µg/g. Blue coloured background gave a carotenoid concentration of 81µg/g. The carotenoid concentration of lobsters reared in black coloured tank was 104µg/g while those of lobsters reared in translucent tank was 95µg/g.

8.1.2. Carotenoid concentration of exoskeleton

Statistical analysis of carotenoid content of exoskeleton of lobsters reared in four different coloured tanks showed significant variations ($P<0.05$). The carotenoid concentration of exoskeleton of lobsters reared in blue coloured tank was 172µg/g. The exoskeleton of lobsters reared in black coloured tank showed the highest carotenoid concentration of 203µg/g. The carotenoid concentration of exoskeleton of lobsters reared in white coloured tank was the lowest (97µg/g) while a concentration of 192µg/g was recorded in lobsters reared in translucent tank. Duncans multiple range test showed highest carotenoid concentration in the exoskeleton of lobsters reared in black coloured tanks followed by translucent, blue and white tanks in that order.

8.1.3. Carotenoid concentration in muscle

The carotenoid concentration in the muscle of lobsters reared in blue tank was 36µg/g while that of lobsters reared in black coloured tank was 34µg/g. White coloured tanks and translucent tanks varied in influencing the

TABLE 18. Carotenoid concentration (μg/g) of different tissues of *P. homarus* reared in coloured tanks

Tissues	Colour of Tanks (Mean ± SD) n=3			
	Black	**Blue**	**White**	**Translucent**
Hepatopancreas	104 ± 2	81 ± 7	45 ± 3	95 ± 2
Exoskeleton	203 ± 7	172 ± 2	97 ± 2	192 ± 3
Muscle	34 ± 2	36 ± 3	25 ± 2	37 ± 2

Table 19 Anova for different coloured tanks

HEPATOPANCREAS

Source of Variation	SS	df	MS	F
Between Groups	5919.3	3	1973.1	77.6
Within Groups	203.3	8	25.4	
Total	6122.7	11		

EXOSKELETON

Source of Variation	SS	df	MS	F
Between Groups	20623.6	3	6874.5	354.1
Within Groups	155.3	8	19.4	
Total	20778.9	11		

MUSCLE

Source of Variation	SS	df	MS	F
Between Groups	248.9	3	83	11.1
Within Groups	60	8	7.5	
Total	309	11		

colour pattern, as the lobsters reared in the white tank had a carotenoid concentration of 25 µg/g while those reared in translucent tank a concentration of 37µg/g. Results of Duncans Multiple range test showed that there was no significant variation in the muscle carotenoid concentration of lobsters reared in black, blue and translucent tanks.

8.2. Discussion

Colour transformations occur due to the chromatophore pigment movements and also due to the quantitative changes either in the number, type or distribution of chromatophores. The carotenoid pigments have been reported to be involved in several protective functions. Smaller crustaceans like *Daphnia* and *Artemia* exhibit orange to red hues, due to the occurence of carotenoid pigments. The intensity of their colouration is related to the concentration of carotenoids which in turn depends upon environmental factors. The general colouration of crustaceans allows them to hide within their surroundings. Deep sea prawns are uniformly red. Red wave lengths of daylight are rapidly absorbed by the surface waters, and only blue and green light penetrate to the depths at which the shrimps live. By absorbing precisely these wavelengths that are available through their red pigment the shrimps become invisible. Only on exposure to daylight, the animals appear intensely red. In contrast, shallow - water crustacea living in coral reefs frequently have bright shining hues, those from rocky and sandy shores are more or less deeply brown and those occurring in seaweeds are green and blue-green (Ghidalia,1985). Colour change is caused by dispersion or concentration of pigment granules within chromatophores. Colour changes of crustaceans in relation to the surroundings

have been well documented. The littoral isopod *Idotea montereyensis* assumes the colour of its algal substrate by gradually incorporating the appropriate carotenoids into its exoskeleton (Lee, 1966). The Pacific mole crab, *Hippa pacifica* develops its cryptic colouration to match a black background in a slow manner, by an increased deposition of black pigments in the exoskeleton (Wenner, 1972), as well as by an increase in the number of epithelial melanophores. The land crab *Gecarcinus lateralis* are red in colour but, their colour changes to grey in conformity with the colour of their substrate. *Crangon vulgaris* changes to conform with white, black, grey, yellow, red and orange backgrounds (Kollen, 1927) and *Palaemon elegans* can match white, black, grey, blue, green, yellow and red backgrounds (Brown, 1935; Robinson and Charlton, 1973). Spiny lobsters which inhabit rocky benthic habitat also exhibits variations in pigmentation based on the habitat.

In less adept species, background adaptations may be restricted to changes in shade from light to dark, causing blanching on whiteground and darkening on black background. These albedo responses, determined by the ratio of reflected to incident light involves pigment movements within one or more types of chromatophores. In species such as *Ligia oceanica, Idotea balthica* and *Squilla empusa* (Fingerman,1969) darkening and blanching are mainly due to melanophore pigment dispersion and concentration respectively. Blanching is usually achieved by pigment dispersion in leucophores and by pigment concentration in dark chromatophores, darkening on the other hand, involves pigment dispersion in dark chromatophores and pigment concentration in leucophores. *Palaemon serratus* retained its pigments when placed on a blackground and lost them on a white background. This is reflected in the

increased number of chromatophores in animals kept on a black background compared with those on a light background (Chassard – Bouchard and Hubert , 1971). This response is mediated by light falling on the eyestalk which act as photoreceptors. In *Dardanus arrosor* illumination stimulates the liberation of hormones which aid in the conversion of B-carotene into astaxanthin. The loss of pigmentation of hypogean species is ascribed to particular conditions like absence of light, low temperature and lack of food (Castillo *et al.*, 1982).

Crustaceans can be classified into two major categories based on their response to the effect of different coloured backgrounds on colouration. The amphipod *Gammarus pulex* (Anders, 1956) and crayfish *Procambarus simulans* (Maguire, 1961) fall in the first category where the colouration of the animal is solely dependent on the genetic constitution of the animal and the supply of dietary carotenoids. The second group embraces all crustaceans where, in addition to the genetic constitution and dietary carotenoids, illumination and background colours have an apparently significant role in controlling pigmentation. The spiny lobster *P. homarus* belongs to this category.

Variations in pigmentation pattern in *P. homarus* reared in different coloured tanks could be ascribed to the influence of environmental factors. Lobsters exhibit background adaptations to colour. The darkest colour was observed in lobsters reared in black coloured tank. The intensity of colour faded in relation to the colour of surroundings. They showed a blanching effect on a white background and darkening effect on a black background. The albedo responses resulted in blanching when melanophore pigment dispersion

occurred. Blackening effect was the result of melanophore pigment concentration. Leucophores and dark chromatophores also controlled the darkening effect. Pigment dispersion in leucophores and pigment concentration in the dark chromatophores produced blanching effect.

The lobsters placed in translucent tank had better colouration when compared with those kept in blue and white coloured tanks. The algal bloom that occurred in the translucent tank could have contributed to the increase in pigmentation pattern. The present study indicates that morphological colour and physiological changes contribute to the pigmentation pattern in lobsters as observed in other crustaceans. Illumination related colour changes involve concentration of chromatophore pigments in darkness and an increased dispersion of chromatophore pigments in relation to the increase in light intensity. These primary responses coupled with background adaptive responses contribute to pigmentation pattern in lobsters (Nagabushanam, 1965; Rao, 1967a; Rao, 1967b; Rao, 1967c; Nagabushanam and Sarojini, 1968, 1969;).

Earlier experiment (Chapter 6) has shown that *P.homarus* exhibited the least body pigmentation when fed with clam *D.cuneatus* which had the lowest carotenoid concentration. The experiment using different coloured tanks clearly indicates that the habitat also has a decisive role in imparting pigmentation to lobsters. The habitat not only influences the distribution or redistribution of chromatophores of *P. homarus* but also the total carotenoid content of various tissues like hepatopancreas, muscle and exoskeleton.

Results of the present study indicate that the right combination of dietary carotenoid with illumination and background colour can play a significant role in controlling pigmentation pattern in *P. homarus*. Dark coloured background result in better pigmentation patterns when compared with light coloured ones. This is because lobsters adapt themselves to the colour of surroundings. Dark coloured background cause darkening of lobsters whereas a blanching effect is seen in lobsters reared in light coloured tanks. Loss of pigmentation can also occur if light is completely cut off from the rearing medium (Castillo *et al.*,1982), the reason could be that light stimulates the liberation of hormones that aid in conversion of β carotene to astaxanthin as reported in *D.arrosor.*

SUMMARY

Crustaceans are incapable of *de novo* synthesis of caroteniods and their carotenoids are of dietary origin. It has been well established that these polyene pigments play an important role in controlling the pigmentation pattern in salmonid fishes. The pink colour of the flesh of salmonids is one of the characteristics of these fishes, which make a major contribution to their elite image. As in the case of salmonids in decapod crustaceans which are marketed live, the colour of exoskeleton is one of the price determining factors. Among decapod crustaceans, lobsters fetch the highest price in the international markets. Lobsters are caught from the wild and are held in fattening ponds since commercial culture practices are not feasible. During fattening, though value addition in terms of increase in size is achieved, a common phenomenon observed was the loss of natural colour pattern. The lobsters held in captivity become pale. This aroused an interest to study the various extrinsic and intrinsic factors which control the pigmentation pattern in lobsters. Studies were also carried out to find out methods of enriching the carotenoid profile of the feeds of lobsters and also the effect of these enriched feeds on the pigmentation pattern in *P. homarus*. The results of carotenoid studies in *P. homarus* are summarized as follows.

1. Moulting, the tegumental rearrangement affects the pigmentation pattern of the animal. It results in loss of pigments via the exuvia, and also through the mobilization of carotenoid pigments to the newly forming teguments.

2. Among the three moult stages studied, carotenoid concentration was the highest in the premoult stages. The lowest carotenoid concentration was in the postmoult stage. Intermoult stages recorded values between premoult and postmoult stages.

3. Carotenoid concentration of hepatopancreas, exoskeleton and muscle tissue during premoult stages were 774μg/g, 725μg/g and 133μg/g respectively. In the post moult stage the carotenoid concentration was 288μg/g in the hepatopancreas, 235μg/g in the exoskeleton and 92μg/g in the muscle.

4. Moulting accounted for 66% loss of carotenoid pigments in *P. homarus.*

5. Astaxanthin levels in *P.homarus* was high in the hepatopancreas and exoskeleton in the premoult stage. Astaxanthin contributed to 60% of the total carotenoids in the hepatopancreas and in the exoskeleton it was 70% of total carotenoids.

6. During postmoult stage the contribution of astaxanthin in the hepatopancreas was 23% of the total carotenoids and in the exoskeleton it was 41%.

7. The increased astaxanthin levels in premoult stage and the drastic decrease in postmoult stage suggests its utilization for the process of ecdysis.

8. During immature stage, the carotenoid concentration was highest in the hepatopancreas (520μg/g). Exoskeleton of immature *P.homarus* had a carotenoid concentration of 330μg/g. The immature ovary had a carotenoid concentration of 128μg/g and was white in colour. The

carotenoid concentration in the ovary increased to 512μg/g and the colour of the ovary was brick red as the lobster matured.

9. The increase in carotenoid concentration in the ovary coincided with the decrease in carotenoid content of the hepatopancreas (320μg/g) and an increase in carotenoid concentration of exoskeleton (472μg/g) This suggests a reproductive function of carotenoids in *P. homarus.*

10. During spent stage an overall decrease of carotenoids was observed in all the tissues studied except for the muscle. Muscle carotenoid concentration was highest in the spent stage (140μg/g). This could be due to resorption of carotenoids from the ovary.

11. Astaxanthin mobilization patterns were similar to those of carotenoids. During early maturation astaxanthin accumulates in the hepatopancreas and during secondary vitellogensis they are mobilized from hepatopancreas to the ovaries. Mobilization of carotenoids occur during maturation and the patterns of mobilization is from hepatopancreas and exoskeleton to the ovaries.

12. Feeds were selected based on the food preferences of lobsters as revealed through gut content analysis. Among the four feeds studied, *(Perna viridis, Donax cuneatus, Paphia malabarica*, *Metapenaeus dobsoni*) the best pigmentation pattern was obtained in *P. homarus* fed on the prawn *M. dobsoni* and green mussel *P. viridis.* The textile clam *P. malabarica* gave better colour patterns than *D. cuneatus* when offered as food to *P. homarus.*

13. In the present study *D. cuneatus was* enriched using *Haematococcus pluvialis* and *Spirulina*. The lobsters fed on the enriched clams were analysed for their carotenoid content. Lobsters fed on *Spirulina* enriched clams had the highest concentration of carotenoids in their exoskeleton (513μg/g) when compared to those fed on the clams enriched with *H.pluvialis* (296μg/g). The control group had a carotenoid concentration of 181μg/g in the exoskeleton.

14. The enrichment studies revealed that *Spirulina* enriched clams were superior to the clams enriched with *H. pluvialis* when given as food to *P. homarus.* The present study reveals that clams, the conventional diet used in fattening purposes can be enriched to achieve better pigmentation pattern in *P. homarus*.

15. Crustaceans exhibit colour patterns which are influenced by environmental factors. These colour changes are displayed not only as an adaptation to match background colouration, but also in response to a wide range of stimuli, including fluctuations in illumination. The present study shows that pigmentation pattern in Indian spiny lobster *P. homarus* under captive conditions is influenced by the colour of rearing tanks. Among the four coloured tanks used (blue, black, translucent and white) the darkest colour was observed in lobsters reared in black coloured tanks. The lobsters reared in translucent tanks had better colouration than those kept in blue and white coloured tanks.

The study in *P. homarus* reveals that a variety of factors govern pigmentation pattern. Colour pattern in lobsters depend on maturation,

moulting, feed and also habitat. While maturation and moulting are biotic factors that cannot be changed, the extrinsic factors namely food and habitat can be manipulated to achieve better pigmentation patterns. The findings of the present study can be applied in fattening. The moult and maturity stages of lobsters have to be determined. Besides these, provision of mussels or prawns which have better carotenoid profiles than clams would help achieve better pigmentation in *P. homarus*. Results of the present study on habitat colouration indicate that dark coloured tanks would be preferred to light coloured ones for pigmentation purposes.

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APPENDIX I

RESULTS OF DUNCANS MULTIPLE RANGE TEST FOR DIFFERENT MOULT STAGES

Homogeneous subsets

Hepatopancreas

MOULT STAGES	N	Subset		
		1	2	3
Postmoult	6	288.3333		
Intermoult	6		328.3333	
Premoult	6			774.6667

Alpha = .05.

Exoskeleton

MOULT STAGES	N	Subset		
		1	2	3
Postmoult	6	235.0000		
Intermoult	6		516.6667	
Premoult	6			725.0000

Alpha = .05.

Muscle

MOULT STAGES	N	Subset		
		1	2	3
Postmoult	6	92.6667		
Intermoult	6		115.166	
Premoult	6			133.8333

Alpha = .05.

APPENDIX II

RESULTS OF DUNCANS MULTIPLE RANGE TEST FOR DIFFERENT MATURITY STAGES

Homogeneous Subsets

Ovary

MATURITY STAGES	N	Subset		
		1	2	3
Spent	6	87.67		
Immature	6		128.07	
Mature	6			512.17

Alpha = .05.

Hepatopancreas

MATURITY STAGES	N	Subset		
		1	2	3
Spent	6	277.07		
Mature	6		320.33	
Immature	6			519.83

Alpha = .05.

Exoskeleton

MATURITY STAGES	N	Subset		
		1	2	3
Spent	6	219.17		
Immature	6		330.00	
Mature	6			471.67

Alpha = .05.

Muscle

MATURITY STAGES	N	Subset		
		1	2	3.
Mature	6	88.03		
Immature	6		124.50	
Spent	6			140.83

Alpha = .05.

APPENDIX III

RESULTS OF DUNCANS MULTIPLE RANGE TEST FOR DIFFERENT FEEDS

Homogeneous subsets

Hepatopancreas

DIET	N	Subset		
		1	2	3
Group A	3	82.0000		
Group C	3		176.6667	
Group B	3			233.3333
Group D	3			263.3333

Alpha = .05.

Exoskeleton

DIET	N	Subset		
		1	2	3
Group A	3	184.6667		
Group C	3		303.3333	
Group B	3			536.6667
Group D	3			546.6667

Alpha = .05.

Muscle

DIET	N	Subset		
		1	2	3
Group A	3	33.6667		
Group B	3	37.3333		
Group C	3		45.3333	
Group D	3			54.3333

Alpha = .05.

APPENDIX IV

RESULTS OF DUNCANS MULTIPLE RANGE TEST FOR ENRICHED FEEDS

Homogeneous subsets

Hepatopancreas

DIET	N	Subset		
		1	2	3
Control Group	3	79.0000		
Group A	3		155.3333	
Group B	3			226.3333

Alpha = .05.

Exoskeleton

DIET	N	Subset		
		1	2	3
Control Group	3	181.0000		
Group A	3		296.6667	
Group B	3			513.0000

Alpha = .05.

Muscle

DIET	N	Subset	
		1	2
Control Group	3	33.0000	
Group A	3	40.6667	
Group B	3		59.0000

Alpha = .05.

APPENDIX V

RESULTS OF DUNCANS MULTIPLE RANGE TEST FOR DIFFERENT COLOURED TANKS

Homogeneous subsets

Hepatopancreas

COLOURED TANKS	N	Subset			
		1	2	3	4
White	3	45.6667			
Blue	3		81.3333		
Translucent	3			94.6667	
Black	3				104.3333

Alpha = .05.

Exoskeleton

COLOURED TANKS	N	Subset			
		1	2	3	4
White	3	95.3333			
Blue	3		172.0000		
Translucent	3			192.0000	
Black	3				202.6667

Alpha = .05.

Muscle

COLOURED TANKS	N	Subset	
		1	2
White	3	25.3333	
Black	3		34.0000
Translucent	3		36.0000
Blue	3		36.3333

Alpha = .05.